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THE APPLE MEALYBUG *PHENACOCCUS ACERIS* SIG. AND ITS CONTROL BY DORMANT SPRAYS[1]

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Two species of mealybug have recently attracted attention as pests of apple in North America. One of these is Comstock's mealybug *Pseudococcus comstocki* Kuw. an insect which was reported by Woodside (3) to be a serious apple pest in some Virginia orchards. The other species, *Phenacoccus aceris* Sig., to which is tentatively applied the name apple mealybug, occurs on a wide variety of plants in Europe, the southern U.S.S.R. and Japan. In North America it was described as a pest of apple by Gilliatt (2) who states that it first became prominent in Nova Scotia in 1932. The only other area of Canada known to be infested is the southern interior of British Columbia and particularly the Kootenay Valley, where it was first reported near Nelson in 1927. By 1936 it was found in orchards at Creston 60 miles east of Nelson and at Robson 30 miles west. A single orchard is known to be infested at Kelowna in the Okanagan Valley.

Comstock's mealybug has several generations a year in Virginia, and winters in the egg stage. The apple mealybug on the other hand, in Canada is single brooded, the males, according to Gilliatt (2), passing the winter in the pupal stage and the females as partly grown larvae. Both sexes are protected during the winter by small white cocoons attached chiefly to the trunks and branches of infested trees. These cocoons may be numerous enough to form a conspicuous white matting.

During the first warm days of February and March and even while snow remains, the immature females leave their cocoons, crawl to twigs and fruit spurs and commence to feed. In the Kootenay Valley the females become mature about the end of April at which time the males emerge from their cocoons and mating takes place. Feeding continues for 2 or 3 weeks before oviposition. The eggs are deposited in masses of from 200 to 500 on twigs, branches or tree trunks. They are protected by white wax filaments that cause the egg mass to resemble an overwintering cocoon although several times larger.

As a rule the eggs do not hatch for 5 or 6 weeks so the first larvae appear about the end of June. They are active and crawl to the undersides of the leaves where they feed for 2 months or longer before migrating to the sheltered positions in which they form cocoons.

Although apple mealybugs may become so numerous that in autumn the leaves and fruits of infested trees are covered with their excretion

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(honeydew), the actual feeding of the insects has not been found to cause obvious damage. But when weather conditions are favourable for the development of sooty fungus, the excretion-covered fruits become blackened and unsaleable unless carefully washed.

Joint experiments on the control of apple mealybug in British Columbia were undertaken by the Dominion and Provincial Departments of Agriculture in 1936. These experiments led to the recommendation of either 6% (actual) dormant oil or a mixture of 4% dormant oil and 6.5% 30-32° Be, lime sulphur. Both spray mixtures were so expensive, however, that there was a noticeable tendency on the part of growers to under-spray. Beginning in 1939 and continuing in 1940 and 1941, orchard experiments were carried on with the object of discovering equally effective but less expensive spray materials. These experiments are summarized below.

MATERIALS AND METHODS

Mature, heavily infested orchards were selected, and experimental plots of 5 and 10 trees laid out with frequent interpolation of check plots. Sprays were applied with a portable machine at the delayed dormant period and after the larval females had left their cocoons and established themselves on the twigs to feed. About 2 months after spraying, 5 terminals each 2 feet long were sampled for records of egg masses or adults.

Available specifications of the substances used in the experiments were:—

Petroleum Oil, E-704, (California crude), Say. Vis. 100° F. 223 sec., U.R. 59%, Dist. at 636° F. 28%.

Petroleum Oil, E-703, (California crude), Say. Vis. 100° F. 108 sec., U.R. 63%, Dist. at 636° F. 50%.

Petroleum Oil, Diesel, (California crude), Say. Vis. 100° F. 44 sec., U.R. 76%.

Petroleum Oil, Stove (California crude), Say. Vis. 100° F. 32 sec., U.R. 80%.

Lime-sulphur, 30-31° Baume, 22% sulphide sulphur.

3, 5–dinitro-o-cresol, M.P. 81.5°–83° C. Soly. (25° C.) .0176 g/100 ml. pH (after 24 hrs.) 3.4, Ash 0.81%.[3]

2, 4–dinitro-6-cyclohexylphenol, fine crystals, no analysis.

Sodium carbonate, anhydrous; sodium hydroxide, technical grade; oxalic acid, technical grade; lignin pitch, hygroscopic sodium material; and sodium lauryl sulphate, commercial grade.

Emulsification of oils was accomplished with lignin pitch. In 1939 it was used at 1 pound and in 1940 and 1941 at ½ pound per 100 Imperial gallons.

RESULTS

Evidently viscosity is an important factor in the toxicity of petroleum oil to the larva of the apple mealybug. An oil with a Saybolt viscosity of 108 seconds at 100° F. was apparently more effective than a more viscous

[3]Analysis by F. A. Herman, Dominion Division of Chemistry, Kentville, N.S.

oil of 223 seconds or less viscous oils of 44 or 32 seconds, the amounts of emulsifier being equal. Although the degree of refinement of the oils was not the same in each instance, it is doubtful if it played an important part in the results. Table 1 indicates the relationship between viscosity and toxicity.

TABLE 1.—EFFECTS OF OILS OF VARYING VISCOSITY ON SURVIVAL OF MEALYBUG

Type of oil	Concentration	Year	Average egg masses or adults 5 terminals per tree
Vis.	%		
223	2	1939	31
108	2	1939	14
223	4	1939	9
108	4	1939	6
Ave. check (no spray)		1939	70
223	2	1940	72
108	2	1940	40
32	2	1940	59
223	4	1940	16
108	4	1940	7
32	4	1940	15
44	6	1940	2
Ave. check (no spray)		1940	89
110	2	1941	11
44	2	1941	23
110	4	1941	2
44	4	1941	6
44	6	1941	1
Ave. check (no spray)		1941	111

From this table it also appears that while an oil of 44 seconds viscosity (Diesel oil) was somewhat less effective than an oil of 108 seconds at an equal concentration, it was as effective at 6% as 108 viscosity oil at 4%. The cost of 6% Diesel oil is but two-thirds as great as 4% 108 viscosity oil which is the standard for dormant use in British Columbia. At 6% concentration, Diesel oil emulsified by 8 ounces lignin pitch per 100 Imperial gallons has caused no apparent injury to apple buds when applied at the delayed dormant period.

A comparison of the effects of petroleum oil and petroleum oil-lime sulphur mixture is given in Table 2. Not revealed by the table, however, is the evidence of 2 years' observations that the oil-lime sulphur mixture has a residual effect that is lacking in oil alone. This desirable quality is offset by the fact that Diesel oil is considerably cheaper than lime sulphur concentrate in British Columbia. Furthermore, the oil-lime sulphur mixture being more objectionable to handle, is likely to be applied less uniformly than oil alone.

TABLE 2.—COMPARISON OF PETROLEUM OIL AND PETROLEUM OIL-LIME SULPHUR AGAINST APPLE MEALYBUG

Type of mixture	Year	Average egg masses or adults 5 terminals per tree
108 Vis. oil 2%	1940	14
108 Vis. oil 2%—Lime sulphur 2%	1940	1
44 Vis. oil 2%—Lime sulphur 2%	1940	2
44 Vis. oil 6%	1940	4
Ave. check (no spray)	1940	70
108 Vis. oil 2%	1941	11
108 Vis. oil 2%—lime sulphur 2%	1941	4
44 Vis. oil 2%	1941	23
44 Vis. oil 2%—lime sulphur 2%	1941	12
44 Vis. oil 4%	1941	6
Ave. check (no spray)	1941	111

The investigation included applications of 3, 5–dinitro-o-cresol and 2, 4–dinitro-6-cyclohexylphenol in oil solution. It was observed that an oil-nitro compound-water-lignin pitch system produced a yellow spray fog. On the other hand, when oxalic acid, 0.25 pound per 100 Imperial gallons, was introduced into the water prior to addition of the solution of oil-nitro compound, the spray was white and indistinguishable from that of an ordinary oil-in-water emulsion. The water before addition of oxalic acid had a pH value of approximately 7.5. After introduction of the acid it was approximately 4.5. It has been pointed out by Boyce, Kagy, Persing and Hansen (1) that oil-nitro compound-water systems are less effective when the water is alkaline than when acid, since at pH values above 5 there is diffusion of the nitro compound from oil to water with the formation of water-soluble salts. There might be several reasons for lowered effectiveness, viz., greater toxicity of parent nitro compound than its salt, better wetting or penetration by oil solution than water solution, greater retention of nitro compound on the sprayed surface when in oil solution. The data in Table 3 support the contention of Boyce *et al.*

TABLE 3.—EFFECTIVENESS OF DINITROPHENOL DERIVATIVES IN OIL SOLUTION

Materials per 100 Imperial gallon	Year	Average egg masses or adults 5 terminals per tree
108 Vis. oil 2 gal.	1939	14
108 Vis. oil 2 gal.—dinitrocresol 6 oz.	1939	9
108 Vis. oil 2 gal.—dinitrocyclohexylphenol 6 oz.	1939	2
Ave. check (no spray)	1939	70
32 Vis. oil 4 gal.	1940	59
32 Vis. oil 4 gal.—dinitrocresol 6 oz.	1940	12
Same but oxalic acid 4 oz. added to water	1940	1
32 Vis. oil 4 gal.—dinitrocyclohexylphenol 6 oz.	1940	5
Same but oxalic acid 4 oz. added to water	1940	3
Ave. check (no spray)	1940	89
44 Vis. oil 2 gal.	1941	23
Check adjoining above plot (no spray)	1941	87
44 Vis. oil 2 gal.—dinitrocresol 3 oz.	1941	53
Same but oxalic acid 4 oz. added to water	1941	15
Check between and adjoining above 2 plots. (No spray)	1941	428

Table 3 indicates further that amounts of dinitrocresol or dinitrocyclohexylphenol of the order of 3 to 6 ounces per 100 Imperial gallons finished spray increase the effectiveness of petroleum oil against apple mealybug.

The effectiveness of dinitrophenol derivatives in water solution was also investigated. Spray solutions were prepared by stirring powdered dinitrocresol or dinitrocyclohexylphenol into a hot water solution of sodium hydroxide or sodium carbonate, then introducing the solution into the spray tank which had previously been filled with water. Sodium lauryl sulphate 1 ounce per 100 Imperial gallons was used as an adjuvant. The comparative toxicities of representative petroleum oil mixtures and sodium dinitrocresylate and sodium dinitrocyclohexylphenate solutions are indicated in Table 4.

TABLE 4.—EFFECTIVENESS OF WATER SOLUBLE DINITROPHENOL DERIVATIVES

Materials per 100 Imperial gallon	Year	Average egg masses on 5 terminals per tree
44 Vis. oil 6 gal.	1940	4
Dinitrocresol 0.6 lb.—sod. carbonate 0.5 lb.	1940	2
Dinitrocyclohexylphenol 0.6 lb.—sod. carbonate 0.5 lb.	1940	0.4
Check (no spray)	1940	81
108 Vis. oil 4 gal.	1941	2
Check adjoining above plot (no spray)	1941	135
Dinitrocresol 0.6 lb.—sod. hydroxide 0.2 lb.	1941	14
Check adjoining above plot (no spray)	1941	357
Dinitrocyclohexylphenol 0.6 lb.—sod. hydroxide 0.2 lb.	1941	0.4
Check adjoining above plot (no spray)	1941	286

These data reveal that dinitrophenol derivatives are highly toxic to larvae of the apple mealybug. Although the sodium salt of dinitrocyclohexylphenol seemed to be slightly more effective than the corresponding salt of dinitrocresol it was more expensive and more objectionable to apply, causing smarting of the mucous membranes.

Evidently satisfactory results against this pest can be obtained by delayed dormant application of a water-soluble dinitrophenol derivative at a cost considerably less than that involved in a petroleum oil application of equal effectiveness.

SUMMARY

1. The apple mealybug, *Phenacoccus aceris* Sig., is a pest of apple occurring in Canada in fruit districts of Nova Scotia and British Columbia. Under certain conditions it causes considerable losses, particularly owing to its excretion serving as a medium for development of sooty fungus on the fruits.

2. Chemical control of the apple mealybug has been most readily and most economically accomplished by use of a dormant or delayed dormant application after the immature females have left their wintering cocoons and moved to the twigs and fruit spurs to resume feeding. In this paper, three years' investigations of such applications are reported.

3. Dormant petroleum oil (California crude) of 108 seconds Saybolt viscosity at 100° F. has been more effective than a more viscous oil of 223 seconds, or less viscous oils of 44 to 32 seconds. From the standpoint of economy, however, an oil of 44 viscosity (Diesel oil) seems preferable to the 108 viscosity type. A 6% application of Diesel oil has been as effective as a 4% application of 108 viscosity oil and costs but two-thirds as much.

4. A mixture of petroleum oil and lime sulphur appears to have a considerable residual effect that is lacking in oil alone. The greater cost of such a mixture, however, appears to outweigh this advantage.

5. Oil solutions containing 1 or 2% dinitrocresol or dinitrocyclohexylphenol were more effective than oil alone. Addition of oxalic acid to lower the pH value of the spray mixture apparently increased the effectiveness of such oil solutions.

6. Water solutions of sodium dinitrocresylate and sodium dinitrocyclohexylphenate proved to be effective against the apple mealybug and less expensive than oil emulsions. The dinitrocyclohexylphenol salt is somewhat objectionable to apply.

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THE EFFECT OF FERTILIZER TREATMENTS ON THE YIELD AND CHEMICAL COMPOSITION OF PASTURE SPECIES[1]

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At the request of the Pasture Committee, Central Experimental Farm, Ottawa, test plots were laid down with the object of studying the effect of applications of nitrogen, phosphoric acid, potash, lime and minor elements such as boron, manganese, copper and sulphur, on the yield and composition of the more common pasture species.

This experiment was started in 1937 at the Division of Forage Plants, Ottawa, Ont., at the Experimental Station, Fredericton, N.B., and at the Experimental Farm, Nappan, N.S. Similar plots were laid down at the Experimental Station at Lennoxville, Que., in 1938.

MATERIALS AND METHODS

The species selected for this experiment were: timothy (*Phleum pratense* L.), Kentucky bluegrass (*Poa pratensis* L.), red top (*Agrostis alba* L.), orchard grass (*Dactylis glomerata* L.), colonial bent grass (*Agrostis tenuis Sibth.*), Canada bluegrass (*Poa compressa L.*), alfalfa (*Medicago sativa L.*), red clover (*Trifolium pratense L.*), alsike (*Trifolium hybridum L.*) and white clover (*Trifolium repens L.*). A nurse crop of barley was used in seeding down all the species in this test.

Only one plot 50 feet long by 6 feet wide of each species was treated with each of the 11 fertilizer treatments listed below. These treatments were randomized independently for each species at each station. There were no replications of the treatments at any one location as it was the intention to use the different stations as replicates of these tests. However, the stands of the species seeded at Lennoxville and Nappan quickly became so impure that the experiments were ploughed up after the first year and the data collected from these two tests were discarded. The stands of Canada bluegrass at Fredericton were impure and yields were not taken from these plots. Orchard grass at that station was completely winter-killed after the first year. It was therefore impossible to combine and analyze the data as originally intended. The data collected at Ottawa and Fredericton were examined separately.

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The fertilizer treatments applied were as follows:

No.	Fertilizer treatments and rates per acre
1	N — Nitrate of soda 150 lbs. before seeding and then annually in early spring.
2	P — Treble super. 100 lbs. before seeding and then annually in early spring.
3	NPK — Nitrate of soda 150 lbs. before seeding and then annually in early spring. Treble super. 100 lbs. before seeding and then annually in early spring. Mur. of potash 100 lbs. before seeding and then annually in early spring.
4	PK — Treble super. 100 lbs. before seeding and then annually in early spring. Mur. of potash 100 lbs. before seeding and then annually in early spring.
5	NPK + Ca — Nitrate of soda 150 lbs. before seeding and then annually in early spring. Treble super. 100 lbs. before seeding and then annually in early spring. Mur. of potash 100 lbs. before seeding and then annually in early spring. + Lime—ground limestone—2 tons applied before seeding only.
6	NPK + Ca + minors — Nitrate of soda 150 lbs. before seeding and then annually in early spring. Treble super. 100 lbs. before seeding and then annually in early spring. Mur. of potash 100 lbs. before seeding and then annually in early spring. + Lime—ground limestone—2 tons before seeding only. + Minor elements Borax—20 lbs. before seeding only. Manganese sulphate 50 lbs. before seeding only. Copper sulphate 10 lbs. before seeding only. Flowers of sulphur 200 lbs. before seeding only.
7	NPK + B — Nitrate of soda 150 lbs. before seeding and then annually in early spring. Treble super. 100 lbs. before seeding and then annually in early spring. Mur. of potash 100 lbs. before seeding and then annually in early spring. + Borax at 20 lbs. before seeding only.
8	NPK + Cu — Nitrate of soda 150 lbs. before seeding and then annually in early spring. Treble super. 100 lbs. before seeding and then annually in early spring. Mur. of potash 100 lbs. before seeding and then annually in early spring. + Copper sulphate 10 lbs. before seeding only.
9	NPK + Mn — Nitrate of soda 150 lbs. before seeding and then annually in early spring. Treble super. 100 lbs. before seeding and then annually in early spring. Mur. of potash 100 lbs. before seeding and then annually in early spring. + Manganese sulphate 10 lbs. before seeding only.
10	Check—no treatment.
11	NPK + S — Nitrate of soda 150 lbs. before seeding and then annually in early spring. Treble super. 100 lbs. before seeding and then annually in early spring. Mur. of potash 100 lbs. before seeding and then annually in early spring. + Flowers of sulphur 200 lbs. before seeding only.

The technique followed in taking yields was to cut the plots with a small garden tractor mower equipped with a centre drive cutting bar arranged in front of the tractor. The cutting bar was a standard type with guards spaced 3 inches apart. A metal pan was attached immediately behind the cutting bar to catch the herbage as it was cut.

All grass plots in the Ottawa test were mown 5 times during 1938 and only 4 times in 1939. The legumes in that test were cut 5 times each season in 1938 and 1939. The species at Fredericton were cut as follows: timothy, Kentucky bluegrass and red top 3 times each season; colonial bent grass was mown only twice each season in 1938 and 1939; alfalfa, alsike and white clover 4 times, and red clover 5 times in 1938 only. All legumes winter-killed at Fredericton in 1938-39.

Green weights of the clipped herbage from each plot were recorded and duplicate 2-pound samples were taken for dry matter determinations. When the total green weight from a plot was 5 pounds or under, all the herbage was taken and dried for yield. The samples obtained from the plots at Ottawa were dried immediately after cutting in an electric dehydrator at 224° F. until constant weight was secured. The samples collected at Fredericton were air-dried at that station and then shipped to the Division of Forage Plants at Ottawa, Ont., for dry matter determinations in the electric dehydrator as above.

The dried samples of herbage from each cutting from each species were then analyzed separately by the Division of Chemistry.

Yields were calculated in pounds of dry matter per acre for each cutting and for the season. All data so collected were statistically analyzed by the variance method but only the summaries are presented in this paper.

Soil from the Experimental Plots

The analysis of the surface 6 inches of soil taken from the experimental areas at Ottawa, Ont., and Fredericton, N.B., are given in Table 1.

TABLE 1.—ANALYSIS OF SOIL AT OTTAWA, ONT., AND FREDERICTON, N.B.

Constituents	Ottawa	Fredericton
Moisture	1.73%	1.72%
Loss on ignition	5.65%	6.67%
Nitrogen	0.24%	0.22%
Phosphoric acid (P_2O_5)		
Total	0.28%	0.25%
Readily soluble	240.0 p.p.m.	212.0 p.p.m.
Exchangeable		
Lime (CaO)	0.23%	0.24%
Magnesia (MgO)	0.047%	0.006%
Potash (K_2O)	0.019%	0.008%
Manganese (as Mn_3O_4)	8.0 p.p.m.	5.0 p.p.m.
Boron Water soluble	0.25 p.p.m.	0.19 p.p.m.
pH	6.09	6.70

The soil at Ottawa was a light clay loam and contained satisfactory amounts of the major elements required for plant growth. The soil at Fredericton was a fine sandy loam which was low in available magnesia and potash. The boron content in these two soils was considered somewhat low.

RESULTS

At the outset, it must be stated that it was very difficult to maintain pure stands of the species sown. Considerable roguing was necessary in all plots. The grass plots were rendered impure by volunteer growth of white clover and alsike. The legumes were seriously encroached upon by indigenous grasses such as bent in the Maritime tests and Kentucky bluegrass at Ottawa.

At Nappan, the stands were so impure that the entire experiment was ploughed up after the first year. The yield data collected during the first year at this location are not included in this report.

At Ottawa and Fredericton reasonably good stands were maintained but not without an enormous amount of roguing. Only the results from these two tests are presented in this paper.

I. Ottawa Test

Grasses

Statistical analysis of the data collected at Ottawa showed that there were no significant differences between the yields of herbage of any one species under different fertilizer treatments. The effects of the fertilizer treatments on the average yields of all grasses is shown in Table 2.

Table 2.—Average seasonal yield and protein content of grasses under different fertilizer treatments, Ottawa, 1938 and 1939

Treatment No.	Fertilizer treatment	Yield per acre		Weighted percentage protein content
		Dry matter	Protein	
		lb.	lb.	%
1	N	1971	282	14.2
2	P	1660	239	14.2
3	NPK	1980	285	14.2
4	PK	1576	227	14.4
5	NPK + Ca	2249	330	15.6
6	NPK + Ca, B, Cu, Mn, S.	2206	319	14.3
7	NPK + B	1937	280	14.4
8	NPK + Cu	1905	274	14.2
9	NPK + Mn	1979	281	14.1
10	Nil	1626	230	14.2
11	NPK + S	1844	257	14.0
Necessary difference for significance at $P = 0.05$		161	22	not significant

It is readily seen that the application of nitrogen significantly increased the yields of dry matter and protein as compared with the untreated plots or those receiving mineral fertilizers only. The two treatments containing lime (Nos. 5 and 6) gave significantly higher yields of dry matter than all other treatments. Treatments P and PK were the only treatments that did not increase the yield of dry matter over the check plots (No. 10).

Table 3 shows the average yields of dry matter, protein, and weighted percentage protein of different species grown under all fertilizer treatments. Orchard grass was significantly higher in yield of dry matter than other grasses, whereas red top gave the highest yield of protein.

The average percentage of protein of all species was not related to the fertilizers applied. In fact, the percentage protein was remarkably constant and not significantly different under the different treatments (Table 2). However, as seen in Table 3, Canada bluegrass was significantly higher in

percentage protein than the other species. When all fertilizer treatments are considered orchard grass had significantly the lowest percentage of protein.

TABLE 3.—COMPARATIVE YIELD AND PROTEIN CONTENT OF PURE SPECIES UNDER ALL FERTILIZER TREATMENTS

Species	Yield per acre		Weighted percentage protein content
	Dry matter	Protein	
	lb.	lb.	%
Timothy	2024	290	14.0
Canada bluegrass	1784	270	15.1
Kentucky bluegrass	1586	230	14.3
Red Top	2346	348	14.6
Colonial Bent grass	1228	183	14.7
Orchard grass	2455	318	12.8
Necessary difference for significance at $P = 0.05$	84	16	0.34

Legumes

Of the four legumes grown under the different fertilizer treatments described above, alfalfa and white clover were harvested in 1938 and 1939, while red clover and alsike were harvested in 1938 only. The average seasonal yields of dry matter and protein and the percentage protein of the legumes under all treatments are given in Table 4.

TABLE 4.—COMPARATIVE YIELD AND PROTEIN CONTENT OF LEGUMES UNDER ALL TREATMENTS, OTTAWA, 1938 AND 1939

Species	Crop years	Yield per acre		Weighted percentage protein content
		Dry matter	Protein	
		lb.	lb.	%
Alfalfa	1938-39	8360	2031	24.2
Wild White Clover	1938-39	1789	500	28.2
Red Clover	1938	5718	1358	23.8
Alsike	1938	3445	800	23.2

Any differences in composition were negligible when compared with the wide differences between yields of species. The white clover was the highest in protein content, but the lowest in yield of dry matter when grown in pure stands.

The data for alfalfa are summarized by treatments in Table 5. There were no significant differences between treatments as measured by yield of dry matter, yield of protein or percentage protein.

TABLE 5.—AVERAGE YIELDS AND PROTEIN CONTENT OF ALFALFA, OTTAWA, 1938 AND 1939

Treatment No.	Fertilizer treatment	Yield per acre		Weighted percentage protein content
		Dry matter	Protein	
		lb.	lb.	%
1	N	7787	1908	24.5
2	P	8407	2002	23.6
3	NPK	8512	2083	24.3
4	PK	8682	2085	24.2
5	NPK + Ca	7702	1892	24.5
6	NPK + Ca, B, Cu, Mn, S.	8902	2230	25.0
7	NPK + B	8672	1967	24.0
8	NPK + Cu	8445	2042	24.0
9	NPK + Mn	9022	2157	24.0
10	Nil	7602	1816	23.7
11	NPK + S	8733	2159	24.9
Necessary difference for significance at $P = 0.05$		not significant	not significant	not significant

Table 6 shows the effect of fertilizer treatments on white clover. It may be seen that the only result of significance was that the percentage protein increased over the no-fertilizer plot when copper or manganese or all the minor elements in addition to NPK were applied.

TABLE 6.—AVERAGE SEASONAL YIELDS AND PROTEIN CONTENT OF WHITE CLOVER, OTTAWA, 1938 AND 1939

Treatment No.	Fertilizer treatment	Yield per acre		Weighted percentage protein content
		Dry matter	Protein	
		lb.	lb.	%
1	N	1437	409	28.6
2	P	1786	490	27.5
3	NPK	1566	442	28.6
4	PK	1813	489	27.0
5	NPK + Ca	1830	520	28.6
6	NPK + Ca, B, Cu, Mn, S.	1891	545	29.1
7	NPK + B	1875	507	27.2
8	NPK + Cu	1804	518	29.0
9	NPK + Mn	1934	557	29.2
10	Nil	1649	455	27.9
11	NPK + S	2099	573	27.4
Necessary difference for significance at $P = 0.05$		not significant	not significant	0.9

There were marked differences in yields of dry matter and protein as well as in percentage protein between fertilizer treatments for red clover

(Table 7). It was impossible, however, to draw any logical conclusions as to the influence of the various plant food constituents applied.

TABLE 7.—SEASONAL YIELDS AND PROTEIN CONTENT OF RED CLOVER, OTTAWA, 1938

Treatment No.	Fertilizer treatment	Yield per acre		Weighted percentage protein content
		Dry matter	Protein	
		lb.	lb.	%
1	N	6006	1399	23.3
2	P	6266	1487	23.7
3	NPK	5344	1281	24.0
4	PK	5139	1272	24.8
5	NPK + Ca	6212	1471	23.7
6	NPK + Ca, B, Cu, Mn, S	5784	1378	23.8
7	NPK + B	6231	1435	23.0
8	NPK + Cu	5750	1364	23.7
9	NPK + Mn	4837	1216	25.1
10	Nil	5985	1365	22.8
11	NPK + S	5341	1265	23.7
Necessary difference for significance at $P = 0.05$		456	84	0.6

The data for alsike are summarized in Table 8. Treatments with B Mn or S with NPK resulted in significantly higher yields of dry matter than those from other treatments. The yield of protein from the NPK + B plot was significantly the highest in the test. The percentage of protein was significantly the highest from the NPK + Ca plot.

TABLE 8.—SEASONAL YIELDS AND PROTEIN CONTENT OF ALSIKE, OTTAWA, 1938

Treatment No.	Fertilizer treatment	Yield per acre		Weighted percentage protein content
		Dry matter	Protein	
		lb.	lb.	%
1	N	3213	759	23.6
2	P	3499	778	22.2
3	NPK	3163	736	23.3
4	PK	3447	808	23.4
5	NPK + Ca	3321	824	24.8
6	NPK + Ca, B, Cu, Mn, S	3534	810	22.9
7	NPK + B	3893	920	23.6
8	NPK + Cu	3077	714	23.2
9	NPK + Mn	3890	866	22.3
10	Nil	3127	734	23.5
11	NPK + S	3733	853	22.9
Necessary difference for significance at $P = 0.05$		278	60	9.7

II. FREDERICTON TEST

Grasses

Only four of the six grass species seeded in 1937 were carried through the crop years of 1938 and 1939. Canada bluegrass was so badly contaminated with volunteer species in 1938 that the results were discarded

and the plots ploughed up. Orchard grass yielded fairly well in 1938, but winter-killed so badly in 1938-39 that the yields for 1939 were not recorded.

The yields of timothy, Kentucky bluegrass, red top and Colonial bent are given in Table 9. Both the dry matter and protein yields were low, particularly in the case of Colonial bent grass. Red top was significantly the lowest in percentage protein.

TABLE 9.—COMPARATIVE YIELD AND PROTEIN CONTENT OF GRASS SPECIES UNDER ALL TREATMENTS, FREDERICTON, 1938 AND 1939

Species	Yield per acre		Weighted percentage protein content
	Dry matter	Protein	
	lb.	lb.	%
Timothy	1094	127	11.5
Kentucky bluegrass	724	97	13.2
Red Top	1330	140	10.5
Colonial Bent grass	473	57	12.0
Necessary difference for significance at $P = 0.05$	94	6	0.3

The average seasonal yields and composition of the four grasses grown under different fertilizer treatments are shown in Table 10. The fertilizer treatments had a significant influence upon the yields of dry matter and protein but not on the percentage protein. The yields of all plots receiving nitrogen were significantly higher than those of no-nitrogen plots. An addition of copper to NPK produced a significant increase in yield over the NPK plots. The addition of lime to NPK caused a significant increase in yield over all other treatments. When minor elements were added to NPK + Ca, the yields obtained were the same as from the NPK plots.

TABLE 10.—AVERAGE SEASONAL YIELDS AND PROTEIN CONTENT OF FOUR GRASSES UNDER DIFFERENT FERTILIZER TREATMENTS, FREDERICTON, 1938 AND 1939

Treatment No.	Fertilizer treatment	Yield per acre		Weighted percentage protein content
		Dry matter	Protein	
		lb.	lb.	%
1	N	981	113	11.7
2	P	603	69	11.8
3	NPK	953	111	12.0
4	PK	604	69	11.8
5	NPK + Ca	1265	156	12.3
6	NPK + Ca, B, Cu, Mn, S	940	107	11.8
7	NPK + B	943	108	11.7
8	NPK + Cu	1109	127	11.6
9	NPK + Mn	977	114	11.8
10	Nil	675	76	11.7
11	NPK + S	910	105	11.9
Necessary difference for significance at $P = 0.05$		156	19	not significant

Legumes

Comparative yields and composition of the four legumes grown at Fredericton in 1938 are given in Table 11. Red clover yielded twice as much dry matter as alfalfa and four times as much as white clover. Alsike was also more productive than alfalfa. White clover was the highest in percentage protein with alfalfa higher than red clover or alsike. A similar observation was made of the legumes grown at Ottawa.

TABLE 11.—COMPARATIVE YIELD AND PROTEIN CONTENT OF LEGUMES UNDER ALL TREATMENTS, FREDERICTON, 1938

Species	Yield per acre		Weighted percentage protein content
	Dry matter	Protein	
	lb.	lb.	%
Alfalfa	2452	706	28.8
Wild white clover	1406	432	30.7
Red clover	5716	1560	27.3
Alsike	3434	940	27.4

The response of alfalfa to the various fertilizer treatments as shown in Table 12 was very erratic. Potassium appeared to be the most beneficial major element applied. Of the minor elements copper with NPK was definitely detrimental to both yield and percentage protein. Copper, however, in combination with other minor elements did not exhibit this effect.

TABLE 12.—SEASONAL YIELDS AND PROTEIN CONTENT OF ALFALFA, FREDERICTON, 1938

Treatment No.	Fertilizer treatment	Yield per acre		Weighted percentage protein content
		Dry matter	Protein	
		lb.	lb.	%
1	N	2282	656	28.7
2	P	2196	647	29.5
3	NPK	2781	811	29.2
4	PK	3051	857	28.1
5	NPK + Ca	3119	891	28.6
6	NPK + Ca, B, Cu, Mn, S	2719	790	29.1
7	NPK + B	2641	755	28.6
8	NPK + Cu	1083	298	27.5
9	NPK + Mn	2209	641	29.0
10	Nil	2114	625	29.6
11	NPK + S	2777	797	28.7
Necessary difference for significance at $P = 0.05$		536	154	0.6

The results for white clover as given in Table 13 were more clear cut. There was a definite beneficial effect from the application of sulphur, while copper and lime were detrimental when applied with NPK. The application of the major elements alone did not increase the yields over the check

plot. Treatments N, NPK + Mn and NPK + S resulted in the highest percentages of protein.

TABLE 13.—SEASONAL YIELDS AND PROTEIN CONTENT OF WHITE CLOVER, FREDERICTON, 1938

Treatment No.	Fertilizer treatment	Yield per acre		Weighted percentage protein content
		Dry matter	Protein	
		lb.	lb.	%
1	N	1152	362	31.4
2	P	1383	423	30.6
3	NPK	1588	478	30.1
4	PK	1341	402	30.0
5	NPK + Ca	1094	328	30.0
6	NPK + Ca, B, Cu, Mn, S	992	301	30.3
7	NPK + B	1405	431	30.7
8	NPK + Cu	969	296	30.5
9	NPK + Mn	1431	448	31.3
10	Nil	1547	476	30.8
11	NPK + S	2562	811	31.7
Necessary difference for significance at $P = 0.05$		411	135	0.6

Table 14 shows the effect of different fertilizer treatments on red clover. It may be seen that NPK + Cu was significantly higher in yield of dry matter than all other treatments except P, and NPK + S. Treatments NPK and NPK + Mn did not give any increase in yield over the check plot. These plots and the one with all minor elements added were also the lowest in yield of protein. No conclusion can be drawn from the weighted percentage protein figures obtained.

TABLE 14.—SEASONAL YIELDS AND PROTEIN CONTENT OF RED CLOVER, FREDERICTON, 1938

Treatment No.	Fertilizer treatment	Yield per acre		Weighted percentage protein content
		Dry matter	Protein	
		lb.	lb.	%
1	N	5844	1620	27.7
2	P	6862	1837	26.8
3	NPK	4980	1387	27.9
4	PK	6242	1720	27.6
5	NPK + Ca	6080	1636	26.9
6	NPK + Ca, B, Cu, Mn, S	4255	1177	27.7
7	NPK + B	5555	1513	27.2
8	NPK + Cu	7262	1975	27.2
9	NPK + Mn	4805	1335	27.8
10	Nil	4515	1220	27.0
11	NPK + S	6489	1740	26.8
Necessary difference for significance at $P = 0.05$		916	241	0.4

The response of alsike to fertilizers is shown in Table 15. The results were almost a direct contrast to those for alfalfa and white clover. The two plots which received copper gave the highest yields of both dry matter and protein. The application of nitrogen alone gave significantly the highest percentage protein.

TABLE 15.—SEASONAL YIELDS AND PROTEIN CONTENT OF ALSIKE, FREDERICTON, 1938

Treatment No.	Fertilizer treatment	Yield per acre		Weighted percentage protein content
		Dry matter	Protein	
		lb.	lb.	%
1	N	3091	909	29.4
2	P	2801	783	28.0
3	NPK	2447	672	27.5
4	PK	4078	1120	27.5
5	NPK + Ca	3243	884	27.3
6	NPK + Ca, B, Cu, Mn, S	4524	1179	26.1
7	NPK + B	2974	839	28.2
8	NPK + Cu	4656	1279	27.5
9	NPK + Mn	3453	912	26.4
10	Nil	2999	792	26.4
11	NPK + S	3509	969	27.6
Necessary difference for significance at $P = 0.05$		665	173	0.9

DISCUSSION

Species

Data for the grasses and legumes grown under all treatments at Ottawa and Fredericton are summarized in Table 16. The grass species at Ottawa yielded approximately twice as much dry matter per acre as those grown at Fredericton. There were even greater differences in the yield of protein since the grasses at Ottawa were from 1 to 4% higher in protein content. Orchard grass was the highest in yield at Ottawa. It was closely followed by red top which was the highest yielding grass species at Fredericton.

TABLE 16.—COMPARATIVE YIELD AND PROTEIN CONTENT OF PURE SPECIES OF GRASSES AND LEGUMES, OTTAWA AND FREDERICTON

Species	Crop year	Yield per acre				Weighted percentage protein content	
		Dry matter		Protein			
		Ottawa	Fredericton	Ottawa	Fredericton	Ottawa	Fredericton
		lb.	lb.	lb.	lb.	%	%
Timothy	1938–39	2024	1094	290	127	14.0	11.5
Canada bluegrass	1938–39	1784	—	270	—	15.1	—
Kentucky bluegrass	1938–39	1586	724	230	97	14.3	13.2
Red Top	1938–39	2346	1330	348	140	14.6	10.5
Colonial Bent	1938–39	1228	473	183	57	14.7	12.0
Orchard grass	1938–39	2455	—	318	—	12.8	—
Alfalfa	1938–39	8360	2452*	2031	706*	24.2	28.8*
White clover	1938–39	1789	1406*	500	432*	28.2	30.7*
Red clover	1938	5718	5716	1358	1560	23.8	27.3
Alsike	1938	3445	3434	800	940	23.2	27.4

* 1938 only.

Red clover and alsike yielded equally well at both stations. Alfalfa at Ottawa, however, was the highest yielding legume tested. This speci yielded poorly at Fredericton.

The legumes grown at Fredericton contained a higher percentage of protein than the legumes at Ottawa. This was in direct contrast with the results for the grasses tested.

Fertilizer Treatments

Grasses—An application of nitrogen resulted in a significant increase in the yield of dry matter and of protein over the plots receiving no nitrogen. Lime in addition to NPK caused a further significant response. An addition of copper to NPK at Fredericton produced a significant increase in the yield of grasses over the NPK plots. Treatments P and PK at Ottawa were the only fertilizers that did not increase the yields over the check plots.

The percentage of protein in grasses did not appear to be related to the fertilizers applied.

Legumes—The results of the experiments with legumes grown under different fertilizers were very erratic and it was impossible to draw any general conclusion from the data obtained. Plots receiving B, Mn, or S in addition to NPK resulted in higher yields of alsike at Ottawa. White clover at Fredericton definitely benefited by the application of sulphur while copper was detrimental to this crop when added to NPK. Alfalfa at Fredericton, grown on the plot treated with NPK + Cu produced significantly the lowest yields of dry matter and protein. Alsike, at this station, however responded well to copper.

No significant differences were observed between the percentage protein in the legumes grown in these two tests.

Effect of Boron and Manganese Treatments

In addition to analysis for protein all the samples of red clover, white clover, timothy and red top grown on plots treated with NPK and NPK + B, at Ottawa and Fredericton, N.B., in 1938 were also analyzed for calcium, phosphorus, manganese and boron. The samples from each cutting were analyzed and the mean seasonal contents calculated as per cent of the seasonal dry matter yields. The summary data so collected are given in Table 17.

TABLE 17.—COMPOSITION OF TWO LEGUMES AND TWO GRASSES GROWN UNDER TWO DIFFERENT FERTILIZER TREATMENTS AT OTTAWA AND FREDERICTON, 1938

Location and species	Treatment NPK				Treatment NPK + B			
	Ca	P	Mn	B	Ca	P	Mn	B
	%	%	p.p.m.	p.p.m.	%	%	p.p.m.	p.p.m.
Ottawa								
Red clover	2.04	0.41	47	25	1.81	0.33	39	26
White clover	1.28	0.39	45	21	1.62	0.42	66	28
Timothy	0.52	0.34	53	12	0.49	0.38	57	15
Red Top	0.66	0.38	157	10	0.71	0.33	154	20
Fredericton								
Red clover	2.57	0.49	90	29	2.18	0.36	85	27
White clover	1.65	0.40	73	27	1.67	0.38	82	29
Timothy	0.51	0.29	58	6	0.46	0.31	84	14
Red Top	0.49	0.25	147	6	0.55	0.25	151	8

An addition of boron, under these conditions had no marked effect upon the calcium and phosphorus contents of the species analyzed. The legumes grown at Ottawa had a lower percentage of calcium than those from Fredericton. The reverse was found for the grasses grown in these two tests.

It was found that manganese content was extremely high in red top, therefore it was decided to examine the species more thoroughly for manganese in the following year. Consequently samples of each cutting from the 1939 crop of all grasses and two legumes grown at Ottawa on plots treated with NPK and NPK + Mn were analyzed for manganese. The results are summarized in Table 18.

TABLE 18.—MANGANESE CONTENT OF GRASSES AND LEGUMES GROWN UNDER TWO DIFFERENT FERTILIZER TREATMENTS AT OTTAWA, 1939

Species	Fertilizer treatment		Means
	NPK	NPK + Mn	
	Mn p.p.m.	Mn p.p.m.	Mn p.p.m.
Timothy	108	138	123
Canada Bluegrass	77	100	89
Kentucky Bluegrass	71	71	71
Red Top	104	153	129
Colonial Bent grass	287	305	296
Orchard Grass	120	133	127
Alfalfa	36	34	35
White Clover	47	53	50
Means	106	123	
Necessary difference for significance at $P = 0.05$		14	28

An analysis of variance of the data for manganese showed that the mean differences between species were highly significant. Colonial bent grass had the highest manganese content. The two legumes were decidedly lower in manganese content than the grasses. With the exception of Kentucky bluegrass, the grasses showed the ability to absorb an increased amount of manganese when it was made available by fertilizer treatment containing this element.

BROWN NECROSIS AND ALTERNARIA BLOTCH OF WHEAT[1]

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INTRODUCTION

For several years dark discolorations have been noted on wheat heads in the field plots of the Dominion Laboratory of Plant Pathology, Winnipeg. Varieties derived from crosses of Marquis and Reward with Hope, H-44, and Pentad have been particularly subject to this condition. The discolorations are usually most pronounced on the outer glumes and on the lemmas, but the rachides, the internodes below each node, and occasionally the upper part of the peduncle, may also exhibit discoloration.

The discoloration on the outer glumes resembles closely that caused by bacterial black chaff—*Xanthomonas translucens* f. sp. *undulosa* (S., J., & R.) Hag.—but frequent failure to isolate the bacterial black chaff organism early indicated some other causal factor. The discoloration on outer glumes, rachides, peduncles, and internodes has been described by McFadden (8) and attributed to infection with stem rust (*Puccinia graminis Tritici* Erikss & Henn.).

The discoloration on the lemmas differs somewhat in appearance from that on the glumes (Figure 1). On the latter it ranges from isolated brown spots to parallel dark-brown stripes co-extensive with the parenchyma tissue between the vascular bundles. On the lemmas, the discoloration, which is grayish-brown or chocolate in colour, is usually more diffuse. In some cases it is confined to irregular brown spots near the tip or base of the lemma, but frequently the entire lemma is discoloured, in which case the floret is generally sterile and the ovary, stigma, and anthers are overgrown with fungal mycelium. This type of discoloration appears first to have been described by Hagborg (4) who found that discoloured lemmas commonly yielded only isolates of a species of *Alternaria* resembling *A. tenuis* Nees. Subsequent isolation studies have shown that, while other fungi are occasionally associated with discoloured lemmas, *Alternaria* is almost invariably present. That the species commonly related to this condition is *A. tenuis* Nees. is indicated by the fact that four cultures isolated from diseased lemmas from three widely separated places in Canada were all determined as this species by Dr. S. P. Wiltshire, Director of the Imperial Mycological Institute, Kew, England.

In certain years, such as 1935 and 1938, discolorations were generally prevalent at Winnipeg on both glumes and lemmas of many newly developed wheat varieties. The fact that these discolorations, and particularly that of the lemma, appeared to be associated with floret

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sterility led to a study of their causes and of the factors favouring their development. It is the object of the present paper to give a brief account of this work.

BROWN NECROSIS OF THE OUTER GLUMES CAUSED BY STEM RUST

McFadden (7, 8) has published data that led him to conclude that stem rust of wheat is capable of causing discoloration, "brown necrosis," on heads, peduncles, and internodes of Hope wheat, and has supplied some evidence to show that the discoloration is most readily effected by rust infection at or shortly after heading.

TABLE 1.—THE RELATION OF WHEAT STEM RUST TO THE DISCOLORATION OF HEADS AND PEDUNCLES OF APEX WHEAT INOCULATED IN THE GREENHOUSE

Treatment	No. heads	No. of discoloured areas on glumes	No. of discoloured lemmas	Average no. discoloured areas per head	No. of discoloured rachides	No. of discoloured peduncles
Experiment 1						
P. gr. Tritici, race 34	12	83	43	10.5	4	4
Control (uninoculated)[1]	14	1	0	0.1	0	0
Control (uninoculated)[2]	12	1	0	0.1	0	0
Experiment 2						
P. gr. Tritici, race 34	12	52	40	7.1	4	4
Control (uninoculated)[1]	12	14	0	1.2	0	0
Control (uninoculated)[2]	11	2	0	0.2	0	0
Experiment 3						
P. gr. Tritici, race 120	10	284	92	37.6	9	6
Control (uninoculated)[1]	11	12	2	1.3	0	0
Control (uninoculated)[2]	11	10	9	1.7	0	0

[1] Placed in damp chambers at the same time as inoculated plants.
[2] Not placed in damp chambers.

As experiments conducted by the writers in the greenhouse and in the field have led to similar conclusions, it seems unnecessary to report these in any great detail. However, as McFadden's data appear to have been drawn largely from observation and experiments carried out in the field, it may be of value to report confirmatory data from greenhouse experiments. In Table 1, three separate infection experiments with newly headed Apex wheat —(H-44 × Double Cross) × Marquis— are summarized. These experiments show clearly the ability of two separate physiologic races of wheat stem rust to cause brown necrosis on glumes, lemmas, rachides, and peduncles of this wheat variety. It will be noted that one of these races (race 120) produced a much greater amount of discoloration than did the other. This difference is apparently associated with the infection characteristics of the races. Race 34 produced pustules of various sizes on the young heads whereas race 120, to which Apex is regarded as being immune, produced no pustules whatever. There seems therefore, in this instance, to be a relationship between the inability of a race to

produce pustules and its ability to produce brown necrosis on the glumes and lemmas. In this connection it may be remarked that, in the field, brown necrosis is rarely found associated with rust pustules.

TABLE 2.—PERCENTAGES OF GLUMES AND LEMMAS DISCOLOURED IN APEX AND RENOWN WHEAT INOCULATED IN THE FIELD AT THREE DIFFERENT STAGES OF HEAD MATURITY WITH *P. graminis Tritici*

Variety and treatment	Stage of head maturity at inoculation		
	Head in boot	Before anthesis	Early milk
	%	%	%
Apex wheat			
Glume discoloration			
Inoculated	**72.5**	43.7	45.5
Not inoculated	29.9	41.5	48.3
Min. Signif. Diff.	9.2	12.5	11.1
Lemma discoloration			
Inoculated	**58.9**	22.8	30.0
Not inoculated	11.5	23.2	25.1
Min. Signif. Diff.	1.7	12.2	8.9
Renown wheat			
Glume discoloration			
Inoculated	**34.6**	18.4	26.1
Not inoculated	12.3	23.4	21.0
Min. Signif. Diff.	8.1	11.7	10.6
Lemma discoloration			
Inoculated	**26.6**	10.4	12.6
Not inoculated	4.9	14.4	14.4
Min. Signif. Diff.	8.9	6.8	6.4

NOTE.—In the first column, the means given are, each, based on the examination of 40 heads, and in the second and third columns, on the examination of 50 heads.
Data that are significantly different from the uninoculated control are indicated in bold-face type.

Attempts have been made to test experimentally the view, expressed by McFadden (8), that stem rust induces brown necrosis most readily in heads that are about to emerge or have just emerged from the boot. Table 2 summarizes a field experiment in which Apex and Renown wheats were inoculated with stem rust at three stages of head maturity: (1) in late boot stage, i.e., just before heading; (2) after heading but before anthesis; (3) about one week after anthesis. In the boot stage, the inoculum was injected into the boot. In the second and third stages, the inoculum was applied by flooding the heads with spore suspensions according to the method devised by Moore (10). In this experiment, only heads inoculated in the boot stage showed significantly greater discoloration than the control heads. Both glumes and lemmas were affected, but discoloration of the latter was chiefly confined to the tip regions.

A second experiment, also performed in the field, produced somewhat different results. Heads of Renown (H-44 × Reward) were dipped in a spore suspension of wheat stem rust at stages of maturity varying from the beginning of heading to the early milk stage, all inoculations being carried out at the same time. As shown in Table 3, all heads that were not past the flowering stage at the time of inoculation showed severe brown necrosis

particularly on the glumes but also to a certain extent on the upper parts of the lemmas.

The results of these experiments would suggest that wheat stem rust may cause discoloration of the glumes and the terminal regions of the lemmas while the plant tissues are still young and succulent, but that ability to cause discoloration diminishes as the tissues grow more mature. This is perhaps the reason for the absence of brown necrosis on Renown, Apex, and other derivatives of Hope and H-44 in certain seasons when stem rust arrives late.

TABLE 3.—DISCOLORATION CAUSED BY *P. graminis Tritici* ON GLUMES AND LEMMAS OF RENOWN WHEAT AT THREE DIFFERENT STAGES OF HEAD MATURITY

Stage of head maturity at inoculation	No. heads	Glumes discoloured	Lemmas discoloured
		%	%
Inoculated heads			
Heading to beginning of anthesis	7	78.0	16.6
Anthesis (early to late)	3	84.0	20.7
Early milk stage	2	7.4	0
Uninoculated heads			
Heading to beginning of anthesis	5	5.2	1.0
Anthesis (early to late)	4	3.8	2.0
Early milk stage	3	0	0

The ability of *P. graminis Tritici* to cause brown necrosis raises the question of the abilities of the other cereal rusts to produce similar symptoms on wheat. Numerous attempts to decide this question by means of infection experiments under greenhouse conditions have thus far indicated that leaf rust of wheat (*Puccinia triticina* Erikss.), stem rust of oats (*Puccinia graminis Avenae* Erikss. & Henn.), and crown rust of oats (*Puccinia coronata Avenae* Erikss. & Henn.) have little, if any, ability to bring about brown necrosis. In 14 separate trials with leaf rust of wheat, only one showed traces of discoloration. In 14 trials with oat stem rust, discoloration was present in only one. As, in this case, considerable brown necrosis resulted on glumes, peduncles, and internodes of the inoculated plants, but not on the uninoculated control plants, it is probable that the symptoms were actually caused by the rust. The negative results of 13 other trials do not suggest that this rust has any pronounced ability to produce brown necrosis. Ten separate infection experiments with crown rust gave negative results. Comparable trials with stem rust of wheat produced brown necrosis in 8 out of 20 trials.

The negative results of most infection experiments with cereal rusts other than wheat stem rust indicate that these rusts are of little, if any, importance in the causation of brown necrosis, but do not definitely exclude the possibility that they may cause some brown necrosis under field conditions.

ALTERNARIA BLOTCH CAUSED BY ALTERNARIA TENUIS

Owing to the constant association of *Alternaria* with the brown discoloration of the lemma, it was obviously necessary to determine experimentally to what extent *Alternaria* isolates from such lemmas were capable of reproducing the symptoms with which they were associated. Greenhouse experiments were suited to the testing of the pathogenicity of *Alternaria* because there the test plants could be kept relatively free from other organisms. Such experiments could not, however, be depended on to give satisfactory information as to the amount of discoloration or floret sterility caused under field conditions, and they were therefore supplemented by field experiments designed to yield information on these points.

Greenhouse Experiments

In the first infection studies carried out in the greenhouse, varieties that had shown severe lemma discoloration in the field were inoculated by dipping the heads in or spraying them with spore suspensions of *Alternaria*, after which the plants were placed in damp chambers for from 24 to 48 hours and then were finally replaced on greenhouse benches. Although experiments of this type were repeated several times at temperatures ranging from 60° F. to 80° F. very few visible infections resulted.

The paucity of infections in these experiments led to a change in the technique of inoculation. Wheat heads were inoculated, before anthesis, by flooding them, under reduced pressure, with spore suspensions according to a method described by Moore (10). This method ensured the introduction of spores between the glumes and within the florets. After inoculation, the plants were replaced on greenhouse benches and subjected for several days to as high atmospheric humidity as could be obtained by spraying the floors, benches, and steam pipes frequently with water. The infection results from one such experiment (Table 4) show that, given favourable conditions, *Alternaria* is capable of bringing about considerable discoloration on Renown wheat.

Table 4.—Discoloration of glumes and lemmas of Renown wheat inoculated with *A. tenuis*

	44 heads inoculated with *Alternaria*	10 heads flooded with water	20 heads left untreated
Number glumes discoloured	507	1	4
Number glumes clean	901	319	634
Per cent glumes discoloured	36.0	0.3	0.6
Number lemmas discoloured	272	0	0
Number lemmas clean	1652	440	872
Per cent lemmas discoloured	14.1	0	0

A similar and simultaneous infection experiment with Marquis wheat also resulted in discoloration, though of a less pronounced character. In this variety the symptoms were entirely confined to the lemmas, 160 out

of 1853 lemmas showing more or less discoloration. No discoloration was noted on the control plants. Isolation studies confirmed the view that *Alternaria* was the cause of discoloration in both varieties.

In the course of infection experiments with *Alternaria* in the greenhouse, the impression was gained that the more closely greenhouse conditions approximated those of the field the more readily was head discoloration obtained. It was supposed that this was due to the presence, in the field, of two factors absent in the greenhouse, (1) a continuous supply of inoculum, and (2) daily opportunity for infection through dew formation at night.

An attempt was therefore made to approximate field conditions more closely during the period of infection than in previous experiments. Heads of C-26-59.2H (Hope × Reward) were flooded, at the beginning of anthesis, with a spore suspension of *A. tenuis*. Thereafter, for 5 days, inoculum was supplied daily by spraying the heads with a spore suspension. During this period the plants were kept, in the daytime, on greenhouse benches at relatively high temperatures while at night they were enclosed in damp chambers at much lower temperatures so as to ensure good dew formation. At the end of this 5-day period the plants were placed on greenhouse benches where they remained until observations were made on discoloration. Control plants were treated in exactly the same way except that no inoculum was supplied. The amount of lemma discoloration secured in two separate experiments is recorded in Table 5.

TABLE 5.—DISCOLORATION CAUSED BY *A. tenuis* ON LEMMAS OF C-26-59.2H (HOPE × REWARD) UNDER CONDITIONS OF DAILY SUPPLY OF INOCULUM AND NIGHTLY DEW FORMATION OVER A 5-DAY PERIOD

Treatment	No. of lemmas		Percentage of lemmas discoloured
	Clean	Discoloured	
Experiment 1			
Spore suspension	598	81	11.9
Water	266	7	2.6
Experiment 2			
Spore suspension	496	52	9.5
Water	314	0	0.0

Although only a relatively small proportion of the lemmas showed brown lesions, the difference between the amount of discoloration in the inoculated and non-inoculated groups demonstrates the ability of *Alternaria* to cause melanistic lesions on the lemmas when greenhouse conditions of inoculum and humidity approximate those commonly present in the field. Yet the low percentage infection would suggest that some other conditioning factor present under field conditions was inoperative in the greenhouse.

FIELD EXPERIMENTS

Field experiments conducted for several years were concerned with the gathering of information on two separate points, (1) the ability of *A. tenuis* to produce discoloration of the lemma, and (2) its ability to cause floret sterility. The latter is discussed elsewhere in this paper.

In these experiments, *A. tenuis* was compared with certain other micro-organisms pathogenic to wheat as to amount of discoloration and type of symptom produced. Inoculations were performed according to Moore's (10) method of flooding the heads with spore suspensions under reduced pressure. In the first two years (1937 and 1938) the heads were to some extent protected against natural inocula of extraneous organisms by covering them with glassine bags as they emerged. These bags remained on the heads to the end of the experiment except for their momentary removal during inoculation. In later experiments the bagging was abandoned owing to the fact that the process was found to bring about considerable floret sterility.

The tests consisted of randomized blocks of 5-foot rows in which there were three or more replicates for each treatment. Sowing was at the rate of about 5 kernels per foot of row, except in 1940 when the rate was doubled.

In the 1937 test, inoculations were made with *Cladosporium herbarum* (Pers.) Lk. as well as *A. tenuis* for the reason that it had been found commonly present in sterile florets with discoloured lemmas. Filtrates from spore suspensions of both organisms were also used for flooding the heads so as to discover if the by-products of the growth of the fungi, on agar, were capable of inducing discoloration. The wheat variety used in this test was C-26-59.2H (Hope × Reward). In this experiment, the spore suspension of *A. tenuis* caused severe discoloration of the lemmas. Isolation studies showed that the cultures of *A. tenuis* recovered from the plants that had been inoculated with that organism differed markedly in growth habit from cultures of *Alternaria* obtained from plants bearing natural infection, but were similar to the cultures used as inoculum. It seems evident, therefore, that the cultures used as inoculum were being reisolated from the inoculated plants. Other treatments, including the spore suspension of *C. herbarum*, did not produce discoloration significantly greater than in the uninoculated control plants.

TABLE 6.—PERCENTAGES OF SPIKELETS WITH ONE OR MORE DISCOLOURED LEMMAS IN C.T. 207 (HOPE × REWARD) INOCULATED WITH SPORE SUSPENSIONS OF FOUR FUNCAL ORGANISMS IN 1938

Treatment	Block I	Block II	Block III	Mean of treatment
A. tenuis suspension	51.2	40.0	40.0	43.7
H. sativum suspension	96.2	87.3	90.9	91.4
F. culmorum suspension	97.0	94.0	92.5	94.5
P. gr. Tritici suspension	6.0	7.1	2.9	5.3
Water only	4.3	5.7	0.0	3.3
Untreated (bagged)	0.9	0.0	1.7	0.9
Untreated (not bagged)	2.7	8.5	10.8	7.3

Minimum significant difference between treatments = 16.2

A similar experiment conducted in 1938 differed chiefly by the inclusion of spore suspensions of *Helminthosporium sativum* P. K. & B., *Fusarium culmorum* (W. G. Sm.) Sacc., and *P. graminis Tritici*. The

results are recorded in Table 6 in which are shown the percentages of discoloured lemmas based on an examination of the lateral florets of 10 spikelets in the middle section of each treated head. Lemma discoloration was caused by *A. tenuis*, *H. sativum*, and *F. culmorum*, but that caused by each organism was characteristic and distinguishable, *Alternaria* causing a rather diffuse grayish-brown discoloration, *H. sativum* a more sharply defined dark-brown or black blotching, and *F. culmorum* a yellowish bleaching occasionally showing some dark pigmentation. No apparent discoloration was produced by stem rust.

The field experiments of 1937 and 1938 had shown that bagging the heads reduced fertility and thereby interfered with the securing of data on the effect of *Alternaria* and other fungi on floret sterility. In later field experiments heads were therefore inoculated without being bagged. Apart from the absence of bagging the technique of inoculation was unchanged. One such experiment, conducted in 1940 and recorded in Table 7, produced rather severe lemma discoloration on the H-44 derivative Renown but only a limited amount on Thatcher, which rarely shows any discoloration of glumes or lemmas in the field. Stem rust appeared to cause no significant lemma discoloration on either variety.

TABLE 7.—PERCENTAGE OF SPIKELETS WITH ONE OR MORE DISCOLOURED LEMMAS IN TWO WHEAT VARIETIES INOCULATED WITH SPORE SUSPENSIONS OF *A. tenuis* AND *P. graminis Tritici* IN 1940

Variety and treatment	Replicates						Mean of treatment
	I	II	III	IV	V	VI	
Renown							
A. tenuis	70	34	63	45	54	55	53.5
P. gr. Tritici	31	4	4	4	8	2	8.8
Water (control)	12	9	2	7	12	15	9.5
Minimum significant difference between treatments = 14.4							
Thatcher							
A. tenuis	11	1	12	8	8	7	7.8
P. gr. Tritici	2	1	4	3	3	0	2.2
Water (control)	1	0	0	1	0	0	0.3
Minimum significant difference between treatments = 6.4							

It is evident from the experiments reported above and from others not recorded here that *A. tenuis* has considerable ability to discolour the lemmas of wheat varieties derived from Hope or H-44. Its ability to discolour the outer glumes is less pronounced, though, in certain experiments, glume discoloration has occurred in the greenhouse and in the field. Only rarely has any indication been obtained of ability to cause discoloration of other plant parts such as rachides, peduncles or internodes, but discolorations of these parts of the plant have not been exhaustively studied. For the symptom caused by *Alternaria* the name *Alternaria* blotch is suggested as the blotchy appearance of the discoloration is characteristic.

FLORET STERILITY

Apart from environmental conditions, which undoubtedly influence the degree of fertility in wheat, the factors most likely to be operative in causing sterility are stem rust and the two fungi (*Alternaria* and *Cladosporium*) that were almost invariably present in the sterile florets. Stem rust has, indeed, been reported by E. C. Johnson (5) as the cause of sterility of wheat in Texas. While numerous references exist to the occurrence of species of *Alternaria* and *Cladosporium* on heads of wheat and of other cereals, these organisms have generally been regarded as saprophytes or, at most, as weak parasites capable only of attacking weakened or dying plant parts. Johnson (5) found *Cladosporium graminum* Cda. associated with spikelet sterility of wheat, but concluded, on the basis of infection experiments, that it was of little or no pathogenic importance. Bennett (1) concluded that *C. herbarum* which is commonly associated with "deaf ears" of wheat in England is not parasitic on cereals, and Bockmann (2) reached a similar conclusion concerning the relation of this fungus to a type of head blackening of cereals prevalent in Germany. *Alternaria*, which likewise was associated with head blackening, was reported by Bockmann to be limited in its parasitism to the production of lesions of microscopic size, any further development being purely saprophytic.

Under Winnipeg conditions, sterility of parasitic origin often was somewhat obscured in some wheats by sterility that was apparently of purely non-parasitic origin. Nevertheless, observational data in several years pointed to a relation between floret sterility and dark discoloration in the heads of some varieties of wheat. This relationship became quite clear when heads of wheat, taken at random in replicated plots, were dissected in detail. Of 1,917 florets of Renown wheat, examined in 1935, 67% were sterile. Of the sterile florets, 47% had discoloured lemmas, while in the fertile florets only 2.5% of the lemmas were discoloured. Similarly, of 6,568 florets of 15 varieties of wheat, examined in 1936, 71% were sterile. Of the sterile florets, 68% had discoloured lemmas, while only 4% of the fertile florets had discoloured lemmas.

The evident association between floret sterility and discoloration led to experiments to determine if inoculation with pathogens that were capable of causing discoloration would also cause sterility. The pathogens tested included *A. tenuis*, *P. gr. Tritici*, *Xanthomonas translucens* f. sp. *undulosa*, and *Pseudomonas atrofaciens* (McC.) Stapp. As *Cladosproium herbarum* had not proved capable of causing discoloration, it was not included in the tests. In greenhouse experiments, little information could be secured owing to difficulties in securing infection. In field experiments, conducted in 1937 and 1938, any increase in sterility, of pathogenic origin, was obscured by the large amount of sterility resulting from the practice of covering the heads with glassine bags to keep out extraneous inoculum, but, in 1939, when the heads were allowed to remain exposed, a field experiment yielded results that were of value.

In the 1939 field experiment, 6 varieties of rust resistant wheat were inoculated, in triplicate plots by Moore's method, with 3 organisms in aqueous suspension, namely, *Puccinia graminis Tritici*, *Alternaria tenuis*, and *Xanthomonas translucens* f. sp. *undulosa*. The control plots were

treated with water only. The inoculations were made shortly after the heads had emerged from the boot, after which the heads were left uncovered until they were harvested in the early dough stage. The data were obtained by counting the number of sterile lateral florets in the 10 median spikelets of each of 10 heads per plot.

By means of the analysis of variance, it was found that significant differences occurred between varieties and between treatments, but not between blocks. As the variance for interaction between varieties and treatments was not significant, the sum of squares for interaction was included with the sum of squares for error. As the F value for blocks was well below the 5% point, the sum of squares for blocks was included with the sum of squares for error in the calculation of the minimum significant difference between treatments. The minimum significant difference between treatments was 13.7 (6.9%). Inoculation with either *Alternaria tenuis* or *X. translucens* f. sp. *undulosa* increased floret sterility significantly but inoculation with stem rust failed to do so (Table 8).

In addition to the significant increase in sterility due to inoculation with either *Alternaria tenuis* or *X. translucens* f. sp. *undolosa*, the differences between varieties were highly significant. An inherent difference between varieties, in the tendency to produce sterile florets, has been noted at this laboratory for many years. The data in the 1939 experiment confirmed these observations.

TABLE 8.—MEAN STERILITY, ACCORDING TO TREATMENT, IN THE LATERAL FLORETS OF SIX RUST-RESISTANT WHEATS FOLLOWING INOCULATION WITH THREE DIFFERENT ORGANISMS IN A REPLICATED FIELD PLOT EXPERIMENT IN 1939

Treatment	Mean sterility, as a percentage of the lateral florets
Puccinia graminis Tritici	19.7
Alternaria tenuis	27.6
X. translucens f. sp. *undulosa*	26.3
Control (water only)	15.7
Minimum significant difference	6.9

In 1940, an experiment with 6 replicates of inoculations with 5 organisms, *Alternaria tenuis*, *Puccinia graminis Tritici*, *Septoria nodorum* Berk., *Xanthomonas translucens* f. sp. *undulosa*, and *Pseudomonas atrofaciens*, was conducted with two varieties, Renown and Thatcher. Although there was a highly significantly greater percentage sterility in Renown than in Thatcher, the sterility data in relation to organism contained no significant differences. Similarly, in 1941, no significant effect on sterility was obtained in inoculations with stem rust at two stages of development after heading, but a significant increase in sterility occurred in the heads inoculated before emergence from the boot. Inoculation with *Alternaria*, in 1941, failed to increase sterility significantly at any of three stages of development.

The somewhat scanty evidence of sterility caused by head discolouring organisms may be due in part at least to weather conditions, in 1938, 1939, and 1940, unsuitable to the development of the type of attack that was so striking in 1935, 1936, and 1937. That this might be the case is suggested in annual readings of the natural attack of black chaff diseases on several varieties of wheat at Winnipeg. In these readings the means for Renown wheat for 1935, 1936, and 1937 was 68% but in 1939, 1940, and 1941, when the experiments on sterility were being conducted, it was only 13.5%.

In view of the fact that statistical proof of sterility due to *A. tenuis* was obtained in only one of three years, and sterility due to *P. graminis Tritici* only following injection into the boot, it is impossible to evaluate clearly what would be the probable effect of the disease factor on sterility under conditions of severe sterility and discoloration, such as occurred in 1935 and to a lesser extent in several other seasons.

MODE OF INFECTION BY ALTERNARIA

It has been stated earlier in this paper that the difficulties of securing infection in greenhouse experiments were partially overcome by flooding the heads, under reduced pressure, with spore suspensions of *Alternaria*. By this process the spores were introduced into the florets where they germinated and occasionally produced on anthers, stigmas, or ovaries, an extensive mycelial growth, which was accompanied by the appearance of discoloured areas on the lemmas.

Observation of the progressive development of the symptoms in the field has shown that frequently the first sign of the disease is a brown lesion below the tip of the lemma. Almost invariably a mycelial growth of *Alternaria* is found on unextruded anthers lodged on the inside at this point. In sterile florets, discoloration appears commonly on the lower part of the lemma, in which case the ovary is usually found to be overgrown with mycelium of *Alternaria*. Discoloration commencing from mycelial growth on the ovary frequently extends over the entire lemma.

Experiments performed in the greenhouse have shown that injured ovaries may serve as infection courts for *Alternaria*. In one such experiment, the ovaries of 98 florets were touched with a hot needle, the heads thereafter being inoculated with a spore suspension of *A. tenuis*. Of the 98 injured florets 73 developed lemma discoloration, while of 64 uninjured, but inoculated, florets only 10 showed discoloration. Examination of the ovaries whether injured or not showed that they were overgrown with mycelium of *Alternaria*. The discoloration on the outer surface of the lemma appeared to originate opposite this mycelial weft.

Injured areas on lemma or glume apparently also provide an infection court for the fungus. In the same experiment, 21 glumes were touched on the margin with a hot needle. Following inoculation, 6 of these developed brown discoloration whereas only 2 of 178 uninjured glumes showed similar symptoms. Although infection may apparently take place in this manner, both observational and experimental evidence indicate that the main course of infection is from spores that gain entrance to the inside of the florets where the presence of dead floral parts, such as anthers or stigmas, favours the establishment of mycelial growth.

HISTOLOGICAL EXAMINATION OF DISCOLOURED TISSUES

Once it was established that *P. graminis Tritici* and *A. tenuis* were capable of causing discoloration, it was obviously necessary to study the process of how discoloration was brought about by these fungi. Histological examination of brown lesions known to have been caused by stem rust and *Alternaria* was therefore undertaken with the purpose of gaining information on the relationship of the fungi to the diseased tissues. Such tissues were examined in various ways, the bulk of the work being done with microtome sections of paraffin-infiltrated glumes discoloured by stem rust and lemmas discoloured by *A. tenuis*. Stains used were chiefly safranin with gentian violet as counterstain, Heidenhain's hematoxylin, and Pianese IIIb.

Examination of glumes of Renown wheat discoloured by stem rust revealed the formation of appressoria and the penetration of entrance hyphae through stomata, but rarely any development of mycelium beyond the substomatal cavity. In discoloured areas, the cell contents of the parenchyma tissue underlying the stomata on the outer side of the glume appeared yellow or brown and were often severely shrivelled. The general appearance suggests that the parenchyma tissue is highly hypersensitive to the rust organism and that, as a consequence, the penetration of the entrance hyphae into the tissue sets up a necrosis which is accompanied by the laying down of a brown, melanin-like pigment. This type of necrosis is distinguished by its brown colour and a tendency to progress considerable distances along the narrow strips of parenchyma tissue between the vascular bundles. The term "brown necrosis" suggested by McFadden (8) appears to be descriptively appropriate.

Examination of lemmas of Apex and Renown discoloured by infection with *A. tenuis* invariably revealed extensive growth of mycelium along the inside surface of the lemma. This mycelial growth had its origin on dead or dying plant parts, such as unextruded anthers and dead or dying stigmas and ovaries. Discoloured areas on the lemmas coincided with the extension of the mycelial growth. In the discoloured tissue, the cell walls and cell contents were charged more or less densely with yellow or brown pigment. Frequently several layers of cells beneath the epidermis were collapsed and compressed to such an extent that the thickness of the lemma was greatly reduced. Despite the abundance of mycelium on the inside surface of the lemma, it was rarely found to have penetrated into the epidermal cells or the tissue beneath them. The fact that mycelium is absent or rarely found in the discoloured tissue suggests that death of the cells results from the action of enzymes or toxins excreted by the mycelium.

DISCUSSION

The fact that stem rust and *A. tenuis* can induce melanistic lesions on Hope, H-44, and many of their derivatives does not signify that all melanism on these wheats is caused by these organisms. On the contrary, there is no doubt but that some of the discoloration present on these varieties in the field is caused by parasitic organisms that produce melanistic lesions on a great variety of wheats—organisms such as *Helminthosporium sativum*,

Xanthomonas translucens f. sp. *undulosa*, *Septoria nodorum*, and others. Furthermore, there is some evidence that melanism occurs spontaneously under certain environmental conditions. Hagborg (4) records the presence of melanistic areas on internodes, peduncles, and rachides of plants kept in damp chambers for periods of from 36 to 60 hours in the absence of inoculation with any pathogen. In various experiments conducted by the writers, melanistic lesions have occasionally developed on uninoculated plants kept in the greenhouse. These lesions were presumably a reaction to environment. No environmental factor yet considered (humidity, temperature, ultra-violet light), however, has consistently induced melanistic symptoms. Nevertheless, the difficulty that has been experienced in inducing discoloration in plants in the greenhouse in winter suggests the probability that light conditions affect the production of melanism, as was thought to be the case for pseudo-black chaff by Broadfoot and Robertson (3). It is evident that Hope, H-44, and many of their rust-resistant derivatives are characterized by the ability to produce melanistic pigment. The nature of the process is not yet understood. It may possibly be conditioned by oxidase action on catechol tannins resulting in the formation of phlobaphenes (6); or, it may be due to the action of tyrosinase on tyrosine and other amino acids, though attempts to induce melanism by the injection of tyrosine and phenylalanine have failed.

It cannot be said that the tendency of certain wheats to develop melanistic areas is of much practical importance. As the discoloration is generally confined to the head and to stem areas just below the nodes, the nodes, the amount of chlorophyllous tissue rendered inactive is relatively small. It is probable that the most important effect of melanistic discoloration is that it may prejudice growers against varieties showing it.

The floret sterility that in some years has been pronouncedly associated with *Alternaria* blotch need not necessarily be a direct consequence of parasitic invasion by that fungus. Certain wheat varieties have a much lower fertility than others, as is shown by a field experiment conducted in 1939 in which the floret sterility in uninoculated control plants ranged from 6% to 38% in the 6 varieties tested. Sterility of this type is probably of chromosomal or genic origin, since varietal differences are pronounced; but environmental influences must exist also since sterility is so much more marked in some seasons than in others. In seasons when floret sterility is high, *Alternaria*, the spores of which are usually present in July and August in great numbers (9), may become established in dead or dying florets and, as a result of mycelial growth within the florets, may bring about much lemma discoloration without being the cause of the sterility. Nevertheless, as is indicated by the experiment recorded in Table 8, *A. tenuis* appears capable under favourable environmental circumstances of causing a material increase in the amount of floret sterility. The stage of development of the floret at the onset of infection is probably an important factor in determining whether or not lemma discoloration is accompanied by sterility. Inoculation of wheat heads after the initation of kernel growth often produces much discoloration of the lemma without any visible effect on the kernel.

It does not appear that lemma discoloration or sterility is caused by special parasitic strains of *A. tenuis*. In a field experiment conducted in

1941, cultures of *A. tenuis* isolated from decaying leaves of oak and hazel produced as much discoloration on Apex and Renown wheats as did strains repeatedly isolated from discoloured lemmas. This fact, together with the absence of any appreciable mycelial invasion of diseased lemmas and failure to increase sterility in the majority of experiments, would indicate that *A. tenuis* is to be regarded at most as a weak parasite.

FIGURE 1. Left—two heads inoculated with *Alternaria tenuis*, showing Alternaria blotch. Right—two heads inoculated with *Puccinia graminis Tritici*, showing brown necrosis. The outer glumes were removed from the two central heads to permit comparison of lemma discoloration.

SUMMARY

It is shown that *Puccinia graminis Tritici* Erikss. and Henn. and *Alternaria tenuis* Nees are capable of producing melanistic discolorations on certain rust resistant wheats derived from crosses with Hope and H-44. Stem rust induced on the outer glumes a discoloration—the "brown necrosis" previously described by McFadden (8)—resembling bacterial black chaff and produced similar symptoms on the tips of lemmas, on rachides, peduncles, and on the internodes immediately below the nodes. Rust infection was found to produce discoloration most readily in the first

few days after heading. Histological examination of discoloured glumes showed penetration of entrance hyphae into the substomatal cavities but no further development.

A. tenuis caused on the lemmas a grayish-brown discoloration, "Alternaria blotch," that resulted from a growth of this organism within the florets. Mycelial growth commonly occurred on unextruded anthers but frequently also involved the ovary, in which case the floret was sterile and the whole lemma discoloured. Lemma discoloration appears to be associated with floret sterility inasmuch as it is far more common in sterile than fertile florets. Cultures of *A. tenuis* isolated from diseased florets have failed to produce floret sterility in the greenhouse but have, in some (but not all) field experiments, caused a significant increase in sterility. A histological study of discoloured lemmas has revealed abundant mycelial growth along the inner (adaxial) surface of the lemma but very rarely any penetration of mycelium into the lemma. The discoloration possibly results from the excretion of toxic substances by the fungal growth within the floret.

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PREDICTION OF MALT DIASTATIC POWER OF HYBRID BARLEYS[1]

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As noted by Sallans and Anderson in reporting their studies on the relationship between barley diastatic power, after activation by papain, and malt diastatic power (5), there is much to be gained in a barley breeding programme if lines can be selected at an early stage on the basis of enzymatic activity. The determination of activated barley diastatic power may be made on a sample of 2.5 grams as compared with a sample for malting of 550 grams. It is evident then that samples can be tested for barley diastatic power much earlier and more conveniently than they can be subjected to malting tests. The studies of Sallans and Anderson showed that between standard varieties and within varieties there were very high correlations between activated barley diastatic power and malt diastatic power. It was suggested that similar studies on hybrid lines were necessary before the prediction method could be introduced as a reliable tool for selecting lines of high enzymatic activity. Determinations of activated barley diastatic power and malt diastatic power have now been carried out on 609 samples from the 1939 crop and 198 samples from the 1940 crop that were submitted for malting tests. The results of the comparisons between these two properties are reported herein. The samples were mainly new hybrids, so that the investigation may be regarded as a test of the utility of the determination of barley diastatic power after activation with papain for predicting malt diastatic power of hybrid samples.

MATERIALS AND METHODS

The samples represented a few standard varieties and a large number of hybrid lines of various origins that have been produced by Canadian plant breeders. There were 16 sets of samples from the 1939 crop and 8 sets from the 1940 crop in this study. The test groups are identified in Table 1. The parentage of the hybrids are of no great consequence, except in a few test groups that will be discussed later. The samples represent a wide choice in parental material and they may be regarded as typical of the barley breeding programmes in progress in Canada at present.

Activated barley diastatic power was determined by the method using papain described by Sallans and Anderson (5). The samples were malted and analysed at the University of Manitoba by the standard procedures used in routine malting tests (1, 2, 4).

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RESULTS AND DISCUSSION

Graphical Representation of Results

The plant breeder is primarily interested in knowing whether the samples selected for high diastatic power by analysis of the barley would also be selected on the basis of malt diastatic power, or, in other words, the percentage selected by both methods. The data for both determinations for the 807 samples studied are given in Figure 1 as a scatter diagram. Without recourse to statistical analysis it is obvious that a close relationship exists between the two properties. In general, the samples that are high in barley diastatic power are also high in malt diastatic power. The results are particularly striking when it is recalled that the samples represent a wide range of environment and that most of the hybrids were grown at only one station.

Assuming that the samples represent one large test group of hybrid lines a stringent test of the efficiency of the selection method based on barley diastatic power may be applied. In Figure 1 a line has been drawn

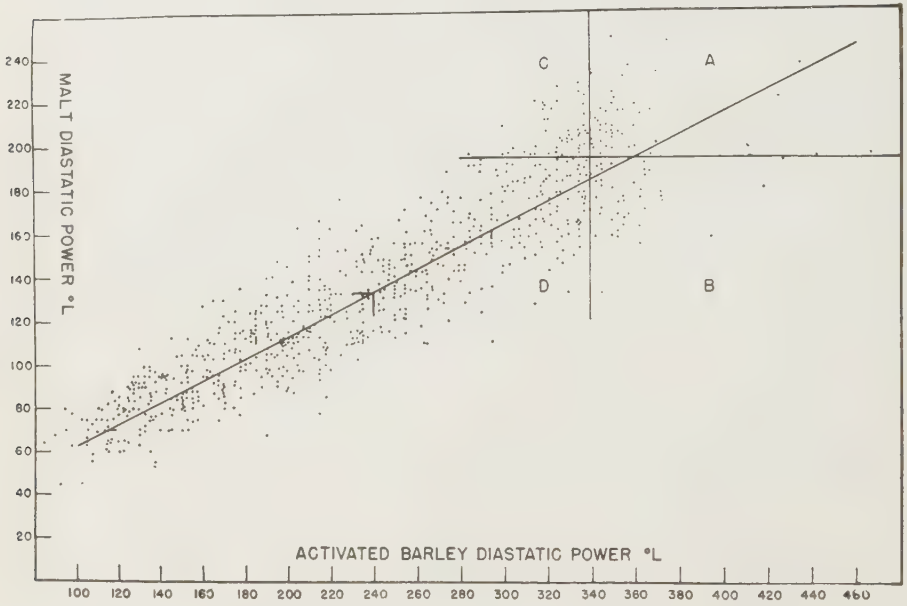

FIGURE 1. Scatter diagram for 807 samples showing relation between diastatic powers of barleys and malts.

separating the 85 samples highest for barley diastatic power from the rest, and another line at right angles to it separates from the rest the 85 samples highest for malt diastatic power. These lines divide the samples into four groups, as follows: Group A, those selected by both methods; Group B, those selected on the basis of barley diastatic power and not by malt diastatic power; Group C, those selected on the basis of malt diastatic power and not by barley diastatic power; Group D, those discarded by both methods.

Group A contains 42 samples and Groups B and C each contain 43 samples. The selection method based on barley diastatic power is therefore

50% efficient, regarding the samples as one test group. This efficiency is much higher than that obtainable by random selection, under which if 80 samples (10% of the total) were selected at random they would contain 8 rather than 40 of the 80 samples highest in malt diastatic power. However, this 50% efficiency is lower than is practically desirable and the reasons for this somewhat low figure were sought. It was found that 25 of the 43 samples in Group B were traceable to one variety, Newal, and hybrids derived from it. These 25 samples originated from three test groups. It is therefore obvious that Newal and its hybrids are different from the other lines and should be regarded as a separate group.

Omitting the Newal lines and again selecting the 85 samples highest for each activity, Group A contains 59 samples so that the general efficiency is 69%. This general efficiency is actually a fair picture of that obtainable by selecting lines with high barley diastatic power, as the weighted mean efficiency within test groups, selecting the top 25% is 68% for the 1939 crop samples and 66% for the 1940 crop samples.

It is apparent that selection of lines of high activated barley diastatic power will enable the plant breeder to segregate a relatively high percentage of those barleys which would produce high malt diastatic powers. The method of selecting those in the highest group is perhaps not as satisfactory as is desirable. On the other hand, the plant breeders usually follow a modified procedure in selecting malting barleys. O.A.C. 21 is the standard

TABLE 1.—IDENTITY OF TEST GROUPS

No.	Test group	No. of varieties	No. of stations	Total no of samples
	1939 Crop			
1	Manitoba strains	19	2	38
2	Manitoba European strains	10	1	10
3	Manitoba secondary test	24	1	24
4	Saskatchewan preliminary test	28	1	28
5	Saskatchewan local test	17	4	68
6	Brandon preliminary test	24	1	24
7	Brandon local test	6	1	6
8	Ottawa Valley trials	12	4	48
9	Macdonald College variety test	23	1	23
10	Ontario Agricultural College strains	10	1	10
11	Eastern hybrids	12	4	48
12	Manitoba co-operative test	12	6	72
13	Macdonald College preliminary test	60	1	60
14	Regional test	12	3	36
15	Alberta variety trials	8	6	48
16	Ontario variety trials	6	11	66
	Total	283	48	609
	1940 Crop			
17	Manitoba strains	24	1	24
18	Saskatchewan strains	72	1	72
19	Manitoba standard strains	12	1	12
20	Manitoba miscellaneous strains	12	1	12
21	Alberta strains	18	1	18
22	Brandon strains	24	1	24
23	Manitoba smooth awned strains	12	1	12
24	Ontario Agricultural College strains	6	4	24
	Total	180	11	198
	Grand Total	463	59	807

malting variety and sets of hybrid lines under test always contain check samples of O.A.C. 21. Examination of the data for the individual test groups showed that no lines that were equal to or greater than O.A.C. 21 in malt diastatic power were lower than it in activated barley diastatic power. Of course quite a number of lines that were equal to or greater than O.A.C. 21 in barley diastatic power were lower than it in malt diastatic power. This method of comparing the activated diastatic powers of hybrid lines with those for O.A.C. 21 will provide a fully reliable measure for selection of lines high in malt diastatic power. Only those lines similar to or higher than the standard in barley diastatic power would be retained. This would provide a set of promising samples, some of which would fail in advanced tests, but the best lines would be retained.

It was pointed out previously that Newal hybrids do not fit into the general picture of the other lines. Their departure from the normal was noted in discussing Figure 1, in that 25 of the 43 samples selected erroneously on the basis of barley diastatic power were Newal hybrids. Figure 2, which presents as a scatter diagram the mean values for each

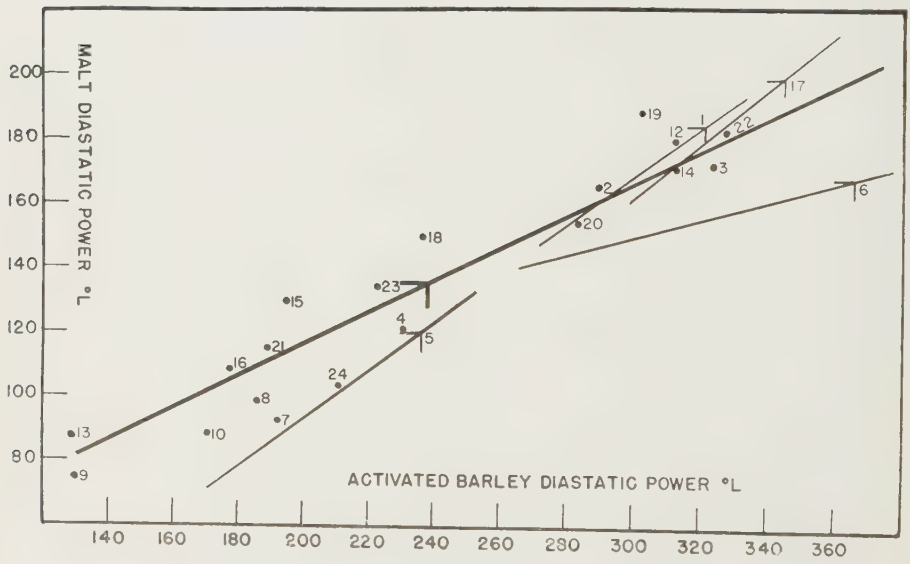

FIGURE 2. Scatter diagram for test group means showing relations between diastatic powers of barleys and malts. The light lines indicate the regression lines that are significantly different from the mean. The key for test groups is given in Table 1.

test group for barley diastatic power and malt diastatic power, shows other abnormalities in certain test groups. Some of the points in the diagram have lines running through them. These will be discussed later and may be disregarded at present. It is readily apparent in Figure 2 that the point for the Newal hybrids, 6, is very far from the general regression line. Considering the remaining points in the upper region of the diagram (assuming that selection for high diastatic power is of greatest interest) it may also be noted that the points for groups 17 and 19 are farthest from

the general regression line. These groups are O.A.C. 21 × Peatland hybrids originated at the University of Manitoba, and their malt diastatic powers are underestimated by the general regression line. These groups, and especially group 19, contribute to a great extent to Group C of Figure 1. The O.A.C. 21 × Peatland hybrids are complementary to the Newal hybrids in abnormality, but another variety that shows abnormalities of the same type as Newal is Byng. Data for this variety are illustrated in Figure 3 which presents scatter diagrams for barley and malt diastatic power for two Ontario variety tests in which Byng was included. In both sections of the figure the point for Byng (3 in Figure 3A and 2 in Figure 3B) is very far from the line common to the other varieties. The malt diastatic powers of Newal and Byng, and hybrids of these, are therefore overestimated and those of O.A.C. 21 × Peatland lines are underestimated when the general relationship between barley diastatic power and malt diastatic power is used for prediction.

It may be concluded that the determination of the activated barley diastatic powers of hybrid lines and comparison of these with the values for comparable samples of O.A.C. 21 provides a reliable basis for selection of lines that will produce high malt diastatic power. However, this prediction method has certain limitations and must be used with due respect for these. Samples of Newal and Byng, and lines derived from these varieties and O.A.C. 21 × Peatland lines, should be segregated from other lines when the prediction method is used. Otherwise, the prediction method based on the general relationship would overestimate by considerable amounts the malt diastatic powers of Newal and Byng lines and underestimate those of O.A.C. 21 × Peatland lines. It appears that Byng and Newal, though high in activated barley diastatic power, are of no great value as parental material for the production of lines of high malt diastatic power.

Statistical Analysis

The determinations of correlation and regression coefficients and other statistics were carried out according to the methods given in detail by Fisher (3) and Snedecor (6).

The general relationships, over all samples within each crop, between the two diastatic powers are given in Table 2, at the top of each crop section. These correlation coefficients are highly significant and demonstrate that a real relationship exists between the two properties, irrespective of variety or place where the samples were grown. The data for each crop were split into component fractions due to relationships within test groups and between test groups and the results are also given in Table 2. That the general relationship holds within individual test groups and between them is shown by these data. The 1939 crop data were split into more fractions than the 1940 crop data as some test groups in 1939 were composed of a series of varieties grown at several stations.

TABLE 2.—RELATIONS BETWEEN ACTIVATED BARLEY AND MALT DIASTATIC POWERS

Relationship determined	Degrees of freedom	Coefficients		Standard error of prediction °L.
		Correlation	Regression m/b	
1939 Crop				
Over all samples	607	.914†	0.48	17
Between test groups	14	.969†	0.47	
Within test groups	591	.812†	0.52	15
Between varieties within stations	513	.747†	0.52	14
Between varieties within test groups	251	.751†	0.50	11
Over all O.A.C. 21 samples	55	.885†	0.48	12
1940 Crop				
Over all samples*	172	.885†	0.50	17
Between test groups	5	.968†	0.49	
Within test groups	166	.803†	0.53	16
Over all O.A.C. 21 samples	15	.475	0.47	17
Within test group 24	22	.331	0.19	
Total				
Over all samples	805	.902†	0.50	18
Between varieties within test groups*	418	.851†	0.50	14

* Test group 24 not included.
† Significant to 1% level.

All correlation coefficients, with the exception of the two at the bottom of the 1940 crop section, are highly significant, but the factor of greatest importance is that all regression coefficients are practically equal. That is, the average relationships between test groups, within test groups and between crops are similar, and the average regression coefficient may be applied to all types of prediction. It is of interest to note that the general prediction equations (which follow) are practically identical.

1. 1939 crop. Malt diastatic power = .48 barley d.p. + 19 (± 17)
2. 1940 crop. Malt diastatic power = .50 barley d.p. + 24 (± 17)
3. Total. Malt diastatic power = .50 barley d.p. + 15 (± 18)

The correlation and regression coefficients between varieties for each test group are given in Table 3. The regression coefficients within each year were compared by means of the *t* test and the coefficients that are significantly different from the mean are marked. In the 1939 crop the regression coefficients for the Manitoba strains, O.A.C. 21 × Peatland lines (test group 1), and the Saskatchewan local tests, also O.A.C. 21 × Peatland parentage (test group 5), are about equal and they are greater than the mean regression coefficient. The coefficient for the Brandon preliminary test, Peatland × Newal lines (test group 6), is lower than the mean. In the 1940 crop only the regression coefficient for the Manitoba

TABLE 3.—RELATION BETWEEN ACTIVATED BARLEY AND MALT DIASTATIC POWERS BETWEEN VARIETIES FOR INDIVIDUAL TEST GROUPS

Test group	No. of varieties	Coefficients		Standard error of prediction °L.	Calculated malt d.p.[1] at barley d.p. of	
		Correlation	Regression m/b		200°L.	300°L.
1939 Crop						
1	19	.809†	.76‡	15	92	168
2	10	.387	.36	16	132	168
3	24	.721†	.54	13	104	158
4	28	.883†	.56	9	104	160
5	17	.898†	.71‡	8	96	167
6	24	.562†	.28‡	17	122	150
7	6	.863*	.52	9	96	148
8	12	.917†	.61	6	106	167
9	23	.831†	.58	8	113	171
10	10	.927†	.45	8	100	145
11	12	.857†	.46	9	116	162
12	12	.439	.44	21	127	171
13	60	.742†	.49	7	123	172
14	12	.760†	.51	15	113	164
15	8	.900†	.54	11	132	186
16	6	.885†	.55	14	120	175
Total and mean	283	.752†	.50	11	114	164
1940 Crop						
17	24	.829†	.82‡	21	78	160
18	72	.819†	.49	15	129	178
19	12	.799†	.41	13	145	186
20	12	.812†	.58	16	106	164
21	18	.868†	.45	10	119	164
22	24	.830†	.54	15	112	166
23	12	.565	.36	12	125	161
24	6	.129	.18	17	101	119
Total and mean§	174	.803†	.53	15	123	176

[1] Necessary difference between test group and mean, 6°L.
* Significant to 5% level.
† Significant to 1% level.
‡ Significantly different, to 5% level, from mean.
§ Test group 24 not included.

strains (test group 17) is significantly different from the mean. It may be noted that test group 17 represents material of the same stock as test group 1 and that test group 19 represents lines selected from test group 1. The strains in test group 18 are derived by selection and breeding from test group 5 and the strains in test group 22 are derived by selection from test group 6. It may be assumed that these selection and breeding programmes are bringing the initial crosses into line with the other lines that show the average regression coefficient. The regression coefficients that differ from the means are shown in Figure 2. The lines for the other test groups are not shown as they may be considered as parallel to the mean regression line.

The significance of these differences between test groups in regression coefficients is not only academic. When predictions are made on the basis of the average regression coefficient, malt diastatic powers of O.A.C.

21 × Peatland lines would be underestimated and those of Peatland × Newal lines would be overestimated. If a test group contained these two types of hybrids the general prediction equation would be of no real value. That this is not merely a theoretical possibility is shown by the results for the Manitoba Co-operative Test. This, test group 12, was composed of equal numbers of O.A.C. 21 × Peatland lines and Peatland × Newal lines. The correlation coefficient between varieties is not significant.

It is therefore evident that in making up samples for prediction tests O.A.C. 21 × Peatland lines and Peatland × Newal lines should be tested separately and not included with other lines.

Further differences between test groups may be observed in the right hand section of Table 3. This gives the calculated values for malt diastatic power for each test group at two levels of barley diastatic power. In particular, it is evident that the Newal lines, test groups 6 and 7, are low in malt diastatic power at the higher level of barley diastatic power. The deficiencies of Newal, and also of Byng, are shown in Table 4, which presents data for the Ontario Variety Trials, 1939 crop. Intra-varietal regression coefficients and malt diastatic power values at the mean barley diastatic power value are given for each of 6 varieties which were grown at 11 stations. Newal and Byng tend to be lower than the other varieties, particularly O.A.C. 21 and Mensury, in regression coefficient. They are also definitely lower than the other varieties in malt diastatic power when comparisons are made at equal barley diastatic power. Figure 3B shows clearly that

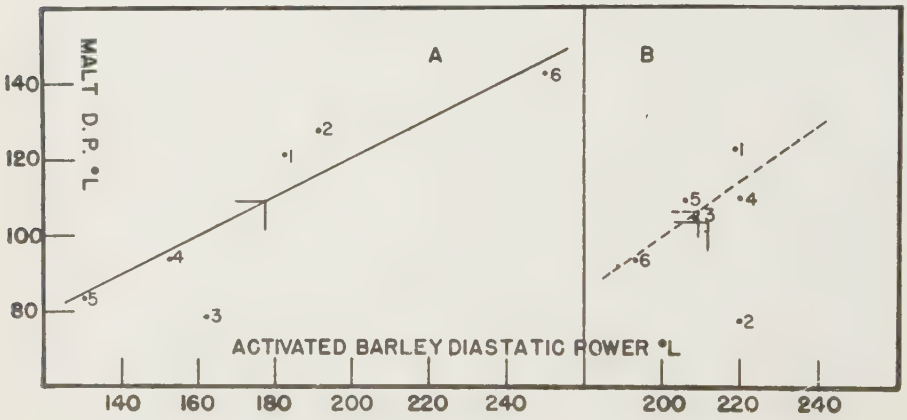

FIGURE 3. Scatter diagrams for varietal means for two Ontario trials showing relations between diastatic powers of barleys and malts.

Key for 3A, test group 16; 1, O.A.C. 21; 2, Mensury; 3, Byng; 4, Nobarb; 5, Regal; 6, Newal.

Key for 3B, test group 24; 1, O.A.C. 21; 2, Byng; 3, Nobarb; 4, 30; 5, 33; 6, 34.

the values for Byng are responsible for the failure of the correlation coefficient for test group 24 (see Table 3) to attain significance.

These results confirm the conclusion given in the previous section that Newal and Byng, and hybrids derived from these varieties, should not be included with other lines in test groups to be submitted to analysis for activated barley diastatic power as a means of selecting the most promising

lines with respect to malt diastatic power. It is also confirmed that O.A.C. 21 × Peatland lines should be kept separate from other lines when making tests, otherwise their malt diastatic powers may be underestimated.

TABLE 4.—RELATION BETWEEN ACTIVATED BARLEY AND MALT DIASTATIC POWERS WITHIN VARIETIES FOR ONTARIO VARIETY TRIALS, 1939 CROP

Variety	No. of samples	Coefficients		Calculated malt d.p.* at barley d.p. of 178°L.
		Correlation	Regression m/b	
O.A.C. 21	11	.949†	0.65	119
Mensury	11	.902†	0.70	120
Byng	11	.934†	0.41	88
Nobarb	11	.950†	0.55	108
Regal	11	.904†	0.55	109
Newal	11	.931†	0.51	100
Mean	11	.920†	0.54	108

* Necessary difference between varieties = 3°L.
† Significant to 1% level.

It may be observed in passing that the standard errors of prediction of malt diastatic power given in Table 3 show that in the majority of test groups the error of estimate is satisfactory. In actual practice, there would be no calculation of malt diastatic power, but the results obtained in these studies suggest that the prediction method may find a place in commercial practice in the selection of samples for special purposes.

General Discussion

The limitations of the prediction method with respect to differences between varieties and between test groups in regression coefficients and malt diastatic power values at equal barley diastatic power are important mainly in an academic sense. The method must be used with care and due respect for its deficiencies. In actual use the limitations are not serious as comparisons with samples of O.A.C. 21 are made within sets grown under the same environment and no comparisons between sets are made. Samples of Newal and Byng may occur in advanced tests along with other varieties but the prediction method would not be used for assessing quality in such tests. The deficiencies of these varieties would be apparent in the results of the malting tests. Hybrids of these varieties may occur in agronomic trials in sets containing other lines. Though it is advisable to keep Newal and Byng hybrids separate from sets of other lines for prediction tests, their inclusion may only cause the retention of poor lines rather than the discarding of promising lines. However, if a large number of lines appeared satisfactory the plant breeder may wish to select only the highest rating samples. Thus Newal and Byng lines may be retained on false premises. This would be prevented by separating such lines when making up sets for

prediction tests. O.A.C. 21 × Peatland lines should also be kept separate from other lines when making up test sets.

It should be observed that it is not proposed to substitute prediction methods for the malting tests, which must remain as one of the final means for assessing malt quality. Prediction methods will be used as a preliminary test before the malting test. By carrying out the determination of activated barley diastatic power on his hybrid lines and comparing the values with those for comparable samples of O.A.C. 21, the plant breeder may obtain useful information on his lines much earlier than could be obtained from malting tests. He would then be able to concentrate on a reduced population of considerable promise and only the better material would be increased and submitted for malting tests.

SUMMARY

Tests were made on the utility of barley diastatic power values, after activation with papain, as a means for selecting hybrid lines with high malt diastatic powers. This determination is made on 2.5 grams of barley and may therefore be carried out at a much earlier stage of production than malting tests. The tests were carried out by determining activated barley diastatic power on 609 samples from the 1939 crop and 198 samples from the 1940 crop that were submitted for malting tests. The resultant data were compared with those for malt diastatic power by means of scatter diagrams. Statistical analyses were also carried out and the results are reported.

It was shown that over all samples there is a very close relationship between the two diastatic powers ($r = .902$ over 807 samples). The regression coefficients between varieties, within varieties and between crops are identical. It is concluded that the determination of activated barley diastatic power of hybrid lines and comparison of the values with those for comparable samples of O.A.C. 21 provides a reliable measure for the selection of those lines that are high in malt diastatic power.

Certain limitations to the prediction method were observed and it is shown that it is advisable to keep O.A.C. 21 × Peatland lines, Newal lines and Byng lines separate from other lines when making up test sets. The malt diastatic powers of the first mentioned lines would be underestimated and those of the Newal and Byng lines would be overestimated by the general prediction method. Differences between test groups in malt diastatic power were also noted when comparisons were made at equal barley diastatic power. These limitations are not serious, as in practice comparisons with O.A.C. 21 are made within test groups grown under the same environment and no comparisons between test groups are made.

ACKNOWLEDGMENTS

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APICAL LEAF SPECK OF POTATOES[1]

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During early August, 1938, and each year since, an apparently unreported nutritional malady has affected the foliage of potato plants on certain low-lying areas in the north and northwestern sections of Edmonton, Alberta, and in several fields just north of the city. All of the affected areas were, prior to cultivation, more or less shallow, local depressions, which held surface water during wet seasons. Although this disorder may prove to be of economic importance to relatively few growers, it seems of sufficient general interest to justify a brief preliminary report now. "Apical leaf speck" is suggested as a suitable name.

DESCRIPTION

In mild cases the colour of the foliage of the potato plant and its yield of tubers may be more or less normal, but when the trouble is pronounced, the vines are less sturdy, the leaves and stems become chlorotic, and the length of the internodes slightly increased. The tubers are normal in shape and usually small. The early appearance of numerous very small, irregular, black dots or specks between the veins on the epidermis of apical leaves (Figure 1A), and later, the development of reddish-brown necrotic areas of various size and shape in the general pith region of the upper half of the stem, are fairly constant diagnostic symptoms. These necrotic areas usually appear at the nodes, as in Figure 1B, but also at random, as in Figure 1C. They develop first toward the top of the stem, are usually absent in the lower half (Figure 1D), and apparently do not form in the stolons or in the main stem below ground level. The affected spots in the stem are often visible in transmitted sunlight. Although the presence of necrotic areas in the pith of the stem is a striking feature of the malady, the specks on the apical leaves are the first external symptoms noticeable. All plants in an affected area develop the typical symptoms. The varieties Netted Gem, Wee McGregor, and Carter's Early Favourite were found to be equally susceptible.

Tests have shown that tubers from affected plants do not perpetuate the disorder. Also, when potato plants were grown in the greenhouse in affected soil, the water content of which was definitely subnormal, typical symptoms were reproduced, but when the same soil was well watered, the pith and apical leaves were apparently normal. Further, there has been no evidence of necrosis in the tubers during storage. This alone would seem to differentiate the present disorder from internal rust spot and spraing (1), or eisenfleckigkeit (2).

Cross-sections through the necrotic areas of the stem illustrated in Figure 1C were examined under the microscope. These areas were found to consist of the pith and contiguous cells with brown walls and contents.

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The cells immediately bordering an affected area were usually colourless. Often one or more of the colourless cells were surrounded by necrotic ones, or *vice versa*.

CAUSE

Considerable study may be required to discover the factors which produce the symptoms just described. However, in the preliminary account it is of interest to mention that the symptoms listed in Chapter 4 of

FIGURE 1. Symptoms of apical leaf speck on potato. A. Dark specks on apical leaf. B and C. Split stems showing necrotic regions each side of nodes and at random. D. Split stems—necrosis in upper half.

Hunger Signs in Crops (3) for deficiency of boron, magnesium, manganese, potassium, phosphorus, or nitrogen do not apply to this particular malady.

The trouble occurred in two contrasting soil types, viz., one high in organic matter, and the other a clay loam, rather deficient in organic matter. The symptoms were more pronounced on the clay loam.

The University of Alberta Department of Soils kindly determined, by the rapid test method, the available phosphorus, potassium, nitrate nitrogen calcium and magnesium, for the two soils, and supplied the following data:

	Organic Loam	*Clay Loam*
	Lbs. A/6 in.	Lbs. A/6 in.
Phosphorus	5 slightly low	2.5—low
Potassium	10—on the line	5.0—low
Nitrogen (NO_3)	10—normal	2.5—low
Calcium	Plenty	Low
Magnesium	7—high	6—high
pH value	6.5—6.9	7.9

Tests for manganese and other elements were not made.

From the results above one cannot obtain a definite clue to the actual cause of the malady. In the organic loam, although only phosphorus was rather low, potassium, nitrogen, and calcium were adequate. However, in the clay loam they were low. Also, there was a difference in pH value of approximately 1. In both soils the plants appeared to grow well until the blossom stage was reached, when the reserve soil water apparently became deficient and the symptoms described appeared.

SUMMARY

A brief description is given of a malady apparently caused by certain soil factors that favour faulty nutrition of the potato plant. Numerous, small black dots on the apical leaves, local necrotic areas in the pith of the upper half of the stem, and the absence of internal necrosis in tubers, or break-down of them in storage, would appear to distinguish this malady from internal rust spot and spraing of potatoes. The name "apical leaf speck" is suggested.

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VARIETAL RESISTANCE OF ALFALFA AND SWEET CLOVER TO ROOT- AND CROWN-ROTTING FUNGI IN ALBERTA[1]

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Root and crown rots of alfalfa and sweet clover are often destructive in Alberta, especially in the early spring. This damage, formerly confused with winter-killing, may be caused by several soil-inhabiting fungi. Some of these pathogens also attack the roots during the growing season, but the damage then is usually much less severe.

Tests for varietal resistance to root- and crown-rotting fungi in alfalfa and sweet clover were started at Edmonton in 1928 by Sanford and Cormack (9), and have been continued every year since. During this time several new pathogens have been found and those of importance have been included in the tests. As soon as they became available, new varieties and strains of the host plants have been tested, and in recent years the experiments have been conducted under different conditions at several points in Alberta.

The first pathogens studied were *Sclerotinia* sp. (recently described as *S. sativa* Drayton and Groves (5)) and *Plenodomus Meliloti* Dearness and Sanford (8). As reported in 1933 (9), varieties apparently differed in their reaction to *S. sativa*, but not to *P. Meliloti*. For this reason the latter was not included in the tests after 1935. In 1937, preliminary results were reported on *Cylindrocarpon Ehrenbergi* Wr. (2), and on *Fusarium avenaceum* (Fr.) Sacc. (3). Experiments with these fungi and with *S. sativa* have been continued, and the data are summarized in the present paper. The results obtained to date with recently discovered pathogens are also included in order to give the plant breeder a more complete picture of the relative resistance of the different varieties to root- and crown-rotting fungi.

IMPORTANCE AND DISTRIBUTION OF THE PATHOGENS

Our knowledge is still far from complete with regard to the relative importance and distribution of the root- and crown-rotting fungi of alfalfa and sweet clover. Considerable information, however, has been obtained from survey and isolation studies carried out for over 12 years in Alberta, and from reports and specimens received from other provinces and regions. The losses vary greatly from year to year, and in different localities, as does also the relative prevalence of the pathogens involved. In the following discussion, the known distribution and host range of these pathogens is briefly summarized, and an estimate is made of the damage which they cause under natural conditions.

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Fungi which Attack Dormant Plants in the Early Spring

The most virulent fungus found on alfalfa is a low-temperature basidiomycete (1), not yet identified, which attacks the crowns and roots about the time of the first spring thaw. Damage ranging from a trace to the destruction of 50% of the plants has been found in many alfalfa fields in central and northern Alberta, and has been reported from the seed-growing areas of Saskatchewan. Sweet clover, *Trifolium* spp., winter wheat, and many grasses have also proved highly susceptible to attack by this pathogen.

Several fungi are associated with early spring damage of sweet clover in Alberta and other parts of Western Canada. *Cylindrocarpon Ehrenbergi* (2) occurs most commonly and may cause death or severe weakening of up to 25% of the plants. *Fusarium avenaceum* (3) and *Plenodomus Meliloti* (8) are usually less prevalent than *C. Ehrenbergi*, but their attack often results in losses ranging from trace to about 10%. *Sclerotinia sativa* (5), although very sporadic in occurrence, occasionally causes up to 50% killing in stands of sweet clover. These fungi also attack alfalfa and *Trifolium* spp. in the early spring, but usually cause relatively slight damage. *F. avenaceum* is known to attack a wide range of plants, including the cereal crops, and *S. sativa* causes a severe bulb rot of tulip and narcissus (5).

Fungi which Attack Growing Plants

Phytophthora Cactorum (Leb. and Cohn) Schroet (4) causes a rootrot of sweet clover which is now quite prevalent in southern Alberta. It may occur at any time during the growing season and causes from trace to 20% mortality in most stands. This disease has also been reported from the United States (7).

Fusarium culmorum (W. G. Sm.) Sacc. (3) sometimes causes severe damage to sweet clover, especially during midsummer. The plants are usually killed in patches, which may involve up to about 10% of the stand. Alfalfa is attacked, but seldom suffers more than slight damage. This widely distributed pathogen is commonly associated with rootrots of the cereal crops.

F. avenaceum and *S. sativa* sometimes attack sweet clover during the growing season, but both are usually less virulent then than in the early spring.

VARIETIES OF ALFALFA AND SWEET CLOVER TESTED

Numerous varieties of alfalfa and sweet clover have been studied during this investigation, but a relatively small number have been included in all experiments. The following varieties have been thoroughly tested for at least three years for resistance to the different pathogens:

Alfalfa

Variegated Group—Baltic, Cossack, Grimm, Hardigan, Ladak, Lytton, Macsel, Ontario Variegated, Viking, and Wisconsin No. 892.

Turkistan Group—Hardistan, Kaw, and Turkistan.

Common Group—Idaho Common, Montana Common, Nebraska Common, and Utah Common.

Yellow-flowered Group—*Medicago falcata.*

Sweet Clover

Melilotus alba—Alpha, Arctic, Brandon Dwarf, Common White, Early Dwarf, Grundy County, and Maccor.

M. officinalis—Albotrea, Common Yellow, Erector, and Zouave.

M. suaveolens—Redfield Yellow.

Some of the above mentioned varieties have not been continued in the tests on account of disease susceptibility, similarity to other varieties, or unsuitable agronomic qualities. For example, Lytton alfalfa was discarded because of extreme susceptibility to downy mildew. All varieties of common alfalfa tested have proved relatively non-hardy and hence unsuitable. As a standard for disease resistance, Grimm alfalfa, Arctic sweet clover, and other commonly grown varieties have been tested every year.

Several new varieties have been tested in recent years. Viking alfalfa is a recently introduced hardy variety developed at the Dominion Forage Crops Laboratory at Saskatoon. Erector sweet clover is a distinct type of *M. officinalis*, and the late-flowering Redfield Yellow is usually referred to as *M. suaveolens*. Of the new strains now being tested, Alpha S-30-35-1-8 is the most promising from the standpoint of root-rot resistance. This strain was originated by Dr. W. J. White at Saskatoon, and consists of the dwarf or Alpha type of progeny from a cross between Alpha 1 and Arctic.

EXPERIMENTAL METHODS

All tests were made in the field using methods similar to those previously reported (9). Each year duplicate experiments were conducted on black soil at Edmonton, and at the Dominion Experimental Station at Lacombe. In addition, similar varietal trials were made at the Dominion Experimental Station at Lethbridge on brown soil, under irrigation, during the seasons of 1938-39 and 1939-40, and at Fallis, 50 miles west of Edmonton, on gray wooded soil during 1939-40. At each location the treatments were made in separate, permanent ranges 5 feet wide and 2 feet apart. Within each range 5 replicate rows of each variety were planted 1½ feet apart in random order. The ranges were cultivated separately by hand and each row was thinned to 20 plants. Thus there were available 100 plants of each variety for inoculation with each pathogen and for the control.

The isolate of each pathogen used for inoculation was the one that had proved most virulent from among a number tested. With most pathogens the plants were inoculated by placing a small portion of oat hull inoculum, on a cotton pad, in contact with each partially bared, but unwounded, tap root. In the case of *Phytophthora Cactorum* mycelial inoculum was inserted beneath the bark of each root. Control plants were similarly treated, except that a sterile medium was employed. After replacement of the soil the plants were left undisturbed until final notes were taken. In the winter tests, the plants were inoculated in the late fall and taken up the following spring, but during the summer the plants were examined about four weeks after inoculation.

The degree of infection of each root was estimated, and expressed by means of the disease rating in percentage previously described (2). The

data were analyzed statistically by Fisher's Analysis of Variance Method (6), using the F test of Snedecor (10) to determine the odds of significance. In this paper a difference between means is considered significant if it exceeds twice the standard error of the difference between two means. This value is referred to as "significant difference" in connection with data for which the F value exceeds the 5% or 1% levels of significance.

EXPERIMENTAL RESULTS

Reaction of Alfalfa Varieties

The low-temperature basidiomycete has proved by far the most virulent of the several pathogens studied in the winter varietal tests with alfalfa, and for this reason it has received the most attention in recent years. Experiments have been continued, however, to verify the results previously reported on some of the other pathogens, namely: *S. sativa* (9), *C. Ehrenbergi* (2), and *F. avenaceum* (3). The results obtained in three winter tests on selected varieties at Edmonton are summarized in Table 1.

Table 1.—Reaction of varieties of *Medicago* to root attack by *Sclerotinia sativa*, *Cylindrocarpon Ehrenbergi*, and *Fusarium avenaceum* in winter tests at Edmonton.

Variety	Disease rating									
	S. sativa				*C. Ehrenbergi*				*F. avenaceum*	Control
	1937–38	1938–39	1939–40	Av.	1937–38	1938–39	1939–40	Av.	Av.	Av.
M. falcata	6.2	11.0	3.6	6.9	4.9	24.6	8.8	12.8	8.6	0.2
Cossack	9.0	28.0	7.6	14.9	14.0	22.4	10.4	15.6	9.4	0.4
Grimm	13.2	39.2	9.4	20.6	16.0	31.0	11.4	19.5	11.6	0.4
Ladak	21.6	40.8	16.1	26.2	18.2	28.4	9.8	18.8	10.2	0.5
Viking	10.4	28.2	12.6	17.1	11.0	29.8	10.2	17.0	12.0	0.6
Hardistan	9.4	—35.6	13.0	19.3	20.0	34.8	15.0	23.3	11.2	1.0
Significant Difference	6.6	7.6	4.4		4.8		3.6			
F Value for Varieties	5.38[2]	7.82[2]	5.18[2]		5.04[2]	2.20	3.33[1]			

[1] Exceeds 5% level of significance.
[2] Exceeds 1% level of significance.

Highly significant differences in varietal reaction occurred in the tests with *S. sativa*. *Medicago falcata* proved extremely resistant to this pathogen, as in previous tests (9), and had the lowest infection rating in all experiments at Edmonton and at the other locations. The variety ranking next was Cossack, with a significantly lower rating than Grimm in the 1938-39 and later experiments. Viking was similar in disease reaction to Cossack in most of the tests. Grimm and Hardistan were in about the same class as Baltic, Ontario Variegated, Turkistan, Wisconsin 892, and other varieties included in the earlier trials. Ladak proved relatively susceptible to *S. sativa* in all experiments.

Alfalfa varieties also differed in their reaction to *C. Ehrenbergi*, but the results were less definite than in the tests with *S. sativa* (Table 1), *Medicago falcata* was relatively resistant, and Hardistan, of the Turkistan group, had a consistently higher infection rating than the varieties belonging to the variegated group. These results confirm those obtained in previous studies (2).

F. avenaceum caused only slight damage to alfalfa, and all varieties were equally resistant. In all cases, however, infection was higher than in the non-inoculated control plants.

Experiments with the Low-temperature Basidiomycete

The varietal tests for resistance to this highly virulent pathogen have not been carried on sufficiently long for conclusive results, and, therefore, only a progress report can be presented at this time. The first experiments were carried out in the winter seasons of 1937-38 and 1938-39 in naturally infested soil of a severely damaged alfalfa field at the Dominion Experimental Station, Lacombe. The varieties were planted in rows 10 feet long in an area where most of the alfalfa plants had been killed. These rows were replicated 6 times in random order and were thinned to 50 plants each. The seedlings grew normally the first season and developed into strong, healthy plants by fall. In the following spring, however, there was a high mortality in nearly all varieties. Although this damage was particularly severe in the early spring of 1938, the results were, unfortunately, ruined by a too high variation between replicates. A more uniform but lower degree of infection was secured the next year in another portion of the field and, as shown in Table 2, there were significant differences in varietal reaction. The least damage occurred in *Medicago falcata* and in the varieties Cossack and Viking. All other varieties tested, except Hardistan, had a relatively high mortality and infection rating.

TABLE 2.—REACTION OF VARIETIES OF *Medicago* TO CROWN AND ROOT ROT CAUSED BY THE LOW-TEMPERATURE BASIDIOMYCETE IN NATURALLY INFESTED SOIL AT LACOMBE, 1938-39

Variety	Disease rating		Plants killed %
	Av.	Range	
M. falcata	23.0	10–100	16
Cossack	30.2	10–100	22
Baltic	43.7	40–100	49
Grimm	48.8	40–100	59
Ladak	44.0	30–100	49
Ontario Variegated	49.0	40–100	61
Viking	32.6	30–100	33
Hardistan	38.3	30–100	47
Significant Difference	11.4		
F Value for Varieties	6.55[1]		

[1] Exceeds 1% level of significance.

Subsequent varietal tests with the basidiomycete have been carried out by artificial inoculation in the field at Edmonton. Under these conditions complete killing of all varieties and strains tested occurred in both

the 1939-40 and 1940-41 experiments. It is hoped that the modified methods of inoculation now being tried will result in a lower degree of infection and thus allow the expression of any existing differences in varietal reaction.

Reaction of Sweet Clover Varieties

Winter Tests with Sclerotinia Sativa

The results of further studies on the susceptibility of varieties of sweet clover to *S. sativa* are in general agreement with those previously reported (9). In four winter tests at Edmonton (Table 3), all varieties of *Melilotus*

Table 3.—Reaction of varieties of *Melilotus* to root attack by *Sclerotinia sativa* in winter tests at Edmonton

Variety	Disease rating					Resistant plants %
	1934-35	1935-36	1936-7	1937-38	Av.	
M. alba						
Alpha	91.2	97.0	94.2	94.2	94.1	0
Arctic	91.0	95.4	90.2	88.8	91.4	0
Common White	91.2	95.2	89.2	97.6	93.3	0
Grundy County	87.2	94.0	93.8	95.6	92.6	0
M. officinalis						
Albotrea	72.4	85.0	73.2	77.4	77.0	4
Common Yellow	76.2	80.4	67.2	74.2	74.5	4
Zouave	76.4	85.2	79.8	86.2	81.9	2
Significant Difference	7.2	6.9	8.1	8.8		

ANALYSIS OF VARIANCE

Variance due to	Degrees of freedom	Mean squares	*F* values obtained	*F* values required	
				5%	1%
Varieties	6	1404.85	42.42	2.19	2.99
Years	3	357.10	10.82	2.70	3.98
Varieties × Years	18	39.10	1.41	1.85	2.37
Replicates	4	46.58			
Error	108	33.12			
Total	139				

alba tested were more susceptible than those belonging to *M. officinalis*. The latter contained a low proportion of apparently resistant plants, which were lacking in the varieties of *M. alba*. Varieties of *M. alba* did not differ significantly in infection rating. In *M. officinalis*, however, Common Yellow and Albotrea usually had a lower rating than Zouave, the disease reaction of which was intermediate. The latter variety, not commonly grown, is more or less intermediate between *M. alba* and *M. officinalis* in growth characteristics.

Similar results were obtained in more recent tests made at different locations in Alberta. In 1939-40 five representative varieties were tested at Edmonton, Lacombe, Lethbridge, and Fallis (Table 4). At all these stations Common Yellow sweet clover had a significantly lower infection rating than the *M. alba* varieties Alpha and Arctic. Erector, however, gave an intermediate reaction in most of the experiments. Redfield Yellow, belonging to *M. suaveolens*, is apparently still more susceptible, since it had a significantly higher rating than Common Yellow at all stations except Lacombe. This confirms the results obtained in experiments at three stations in 1938-39 in which Redfield Yellow reacted similarly to the *M. alba* varieties.

TABLE 4.—REACTION OF VARIETIES OF *Melilotus* TO ROOT ATTACK BY *Sclerotinia sativa* AT FOUR STATIONS IN ALBERTA. (WINTER TESTS, 1939-40)

Variety	Disease rating					Resistant plants %
	Edmonton	Lacombe	Fallis	Lethbridge	Av.	
M. alba						
Alpha	100.0	99.0	98.6	90.3	97.0	0
Arctic	99.2	98.4	95.2	84.8	94.4	0
M. officinalis						
Common Yellow	94.8	88.8	84.0	71.2	84.7	4
Erector	97.6	94.4	90.3	71.4	88.4	3
M. suaveolens						
Redfield Yellow	98.6	94.8	94.2	77.4	91.2	1
Significant Difference	3.0	9.3	7.3	5.6		

ANALYSIS OF VARIANCE

Variance due to	Degrees of freedom	Mean squares	*F* values obtained	*F* values required	
				5%	1%
Varieties	4	1388.69	17.08	2.49	3.56
Stations	3	447.88	5.51	2.72	4.04
Varieties × Stations	12	35.26			
Replicates	4	73.92			
Error	76	81.28			
Total	99				

The analyses given in Table 3 show that highly significant differences also occurred from year to year in the degree of infection caused by *S. sativa*. For example, the infection ratings were generally higher in the 1935-36 experiment at Edmonton than in the 1934-35 and 1936-37 tests. However, the interaction of varieties and years was not significant, since the *F* value did not exceed the 5% point. These results indicate that the relative degree of infection of the different varieties was similar from year to year. Hence the data obtained in each experiment can be considered as a reasonably reliable criterion of varietal reaction to this pathogen.

Highly significant differences in degree of infection also occurred at the different locations in the 1939-40 experiments, as shown by the relative magnitude of the *F* values given in Table 4. There was no interaction, however, between varieties and stations. In spite of the fact that infection was generally higher at Edmonton and Lacombe than at either Lethbridge or Fallis the varieties reacted similarly at all stations. This indicates that varying soil and climatic conditions do not have an important influence on the reaction of sweet clover varieties to *S. sativa*.

Winter Tests with Other Pathogens

C. Ehrenbergi was moderately virulent on all sweet clover varieties tested in three experiments at Edmonton (Table 5). As in previous studies (2), there were no consistent differences in reaction between varieties of *M. alba* and *M. officinalis*. Redfield Yellow and Arctic usually had a lower infection rating than the other varieties, but the differences were significant in only one of the tests (1939-40). Similarly, in experiments carried out at three other stations in Alberta, the varietal differences were significant only in the 1938-39 test at Lacombe, where Redfield Yellow and Arctic were again less severely attacked than the other varieties.

TABLE 5.—REACTION OF VARIETIES OF *Melilotus* TO ROOT ATTACK BY *Cylindrocarpon Ehrenbergi* AND *Fusarium avenaceum* IN WINTER TESTS AT EDMONTON

Variety	Disease rating								
	C. Ehrenbergi				*F. avenaceum*				Control Av.
	1937-38	1938-39	1939-40	Av.	1937-38	1938-39	1939-40	Av.	
M. alba									
Alpha	50.8	46.2	41.0	46.0	30.8	35.8	17.2	27.9	2.3
Arctic	32.8	40.4	37.0	36.7	27.8	33.4	11.0	24.1	4.5
Grundy County	44.0	43.8	45 6	44.5	30.2	32.8	15.1	26.0	2.6
M. officinalis									
Common Yellow	44.6	36.2	41.0	40.6	25.2	39.0	12.2	25.5	2.8
M. suaveolens									
Redfield Yellow	37.4	32.0	29.2	32.9	23.0	23.2	10.8	19.0	3.4
Significant Difference			6.2			7.8			
F value for varieties	2.60	1.23	7.75[2]		1.11	4.61[1]	2.98		

[1] Exceeds 5% level of significance.
[2] Exceeds 1% level of significance.

Even less marked varietal differences appeared in the case of *F. avenaceum*. The *F* value for varieties exceeded the 5% point of probability in only two trials, namely: Edmonton in 1938-39 (Table 5), and Lacombe in 1939-40. In both cases Redfield Yellow had a significantly lower infection rating than the other varieties tested.

Sweet clover varieties have also been tested for resistance to the virulent low-temperature basidiomycete isolated from alfalfa (1). As with alfalfa, the first tests were made in 1937-38 and 1938-39 in naturally infested soil at Lacombe on all available varieties. In both experiments the

infection ratings ranged from 75 to 95%, but there were no significant differences in varietal reaction. Since then complete killing of all varieties and strains has occurred in the artificial inoculation tests at Edmonton. These experiments are being continued, under less severe conditions, in the hope that partially resistant varieties or strains may be found.

Summer Tests with Phytophthora Cactorum and Fusarium Culmorum

Varieties of sweet clover differed markedly in their reaction to *P. Cactorum* in experiments recently carried out at Edmonton (Table 6). In the first test, made on 1-year-old plants in May, Alpha S 30-35-1-8 showed outstanding resistance, and the original Alpha also had a significantly lower infection rating than any of the other varieties tested. Arctic and Grundy County were highly susceptible, as in preliminary tests with these varieties, and the yellow-flowered varieties, with the exception of Erector, were intermediate in reaction. These differences are indicated by the infection rating data, with an *F* value exceeding the 1% point, and also by the percentages of highly resistant and dead plants in each variety, as given in Table 6. Similar results were obtained by inoculation of 3-month-old plants of a few of these varieties (Table 6). The Alpha strain contained about 62% of highly resistant plants, as compared to only 3% in the commonly grown Arctic and Common Yellow varieties. Selection work and further varietal tests for resistance to this pathogen are now in progress.

TABLE 6.—REACTION OF VARIETIES OF *Melilotus* TO ROOT ATTACK BY *Phytophthora Cactorum* AND *Fusarium culmorum* DURING THE GROWING SEASON (EDMONTON, 1941)

Age	Variety	Inoculum			
		P. Cactorum			*F. culmorum*
		Highly resistant %	Dead %	Disease rating[1]	Disease rating[2]
1 year May, 1941	*M. alba*				
	Alpha (S 30-35-1-8)	50	18	50.8	45.3
	Alpha (Original)	36	41	63.4	48.8
	Arctic	10	79	88.6	49.0
	Grundy County	2	86	90.6	43.0
	M. officinalis				
	Albotrea	15	63	77.6	44.8
	Common Yellow	20	57	74.2	46.8
	Erector	10	73	84.8	49.8
	M. suaveolens				
	Redfield Yellow	14	56	74.2	45.0
3 months Sept., 1941	Alpha (S 30-35-1-8)	62	0	23.4	
	Arctic	3	43	65.0	
	Yellow Blossom	3	42	63.4	

[1] *F* values exceed 1% level of significance. Differences required for significance = 5.5 and 9.8 for the 1-year and 3-months old plants, respectively.
[2] No significant differences between varieties.

In experiments with *F. culmorum* all varieties of sweet clover tested were attacked with approximately equal severity. For example, in the 1941 summer test at Edmonton (Table 6), the *F* value for varieties did not

exceed the 5% level of significance. Similar results were obtained in previous tests on growing plants of these and other varieties.

DISCUSSION

Although this study is not yet complete it has already provided data of much value to any programme for breeding alfalfa and sweet clover more resistant to the root-rotting pathogens. Also, a much clearer idea of the relative pathogenicity and economic importance of the various fungi involved has been made available.

The results obtained with alfalfa indicate that *Medicago falcata* possesses a considerable degree of resistance to *S. sativa*, *C. Ehrenbergi*, and *F. avenaceum*. The variety Cossack ranked next to *M. falcata* in resistance to most of the pathogens, and generally proved less susceptible than Grimm. The recently introduced variety Viking reacted similarly to Cossack in several experiments, but requires further testing.

In sweet clover, varietal resistance to one pathogen was seldom correlated with resistance to other pathogens. For example, most varieties of *Melilotus officinalis* proved slightly more resistant to *S. sativa* than those belonging to *M. alba*, but varieties of these two species showed no consistent differences in reaction to *C. Ehrenbergi*. In *M. officinalis*, resistance to *S. sativa* was most evident in varieties with a prostrate habit in the first year of growth, eg., Albotrea and Common Yellow. The varieties Zouave and Erector, with more erect growth, were also more or less intermediate to *M. alba* in their reaction to *S. sativa*. Redfield Yellow, belonging to *M. suaveolens*, was almost as susceptible as the varieties of *M. alba* in tests with *S. sativa*, but it appeared slightly resistant to *C. Ehrenbergi* and *F. avenaceum*. Despite the expected yearly fluctuation in severity of disease caused by *S. sativa* at different locations, the relative position of the varieties was not affected.

The most outstanding case of resistance encountered in any of the tests was that of Alpha S 30-35-1-8 sweet clover to *Phytophthora Cactorum*. This strain apparently contains 50% or more of highly resistant plants, as compared to about 35% in the original Alpha, and 20% or less in the other varieties tested. Jones (7) also found plants resistant to Phytophthora rootrot in certain strains of sweet clover and by selection and selfing obtained progenies with 50 to 75% of healthy plants. Similar selection studies with Alpha S 30-35-1-8 are now in progress here. It was hoped that this Alpha strain would also prove relatively resistant to other pathogens. Preliminary tests indicate that it may be somewhat resistant to *S. sativa* and *F. avenaceum*, and that it reacts in about the same way as Arctic and other varieties to *C. Ehrenbergi* and *F. culmorum*.

With regard to the low-temperature basidiomycete, the results indicate that it may be difficult to discover much resistance in any of the varieties of alfalfa, sweet clover, other legumes, or grasses. This, of course, emphasizes the necessity of making every effort to secure strains more resistant to this highly virulent pathogen.

The work of securing greater varietal resistance to the various pathogens is now being carried out in co-operation with the Dominion Forage Crops Laboratory at Saskatoon, Saskatchewan, which laboratory attends to the plant breeding phases. Resistance trials and other phytopathological aspects will be continued by the Dominion Laboratory of Plant Pathology, Edmonton, Alberta.

SUMMARY

The results of field tests during seven years, to determine the resistance of all available hardy varieties and strains of alfalfa and sweet clover to six root- and crown-rotting fungi in Alberta are reported.

On the basis of present information, the pathogens attacking alfalfa and sweet clover during early spring rank, in order of destruction caused, as follows: the low-temperature basidiomycete, *Cylindrocarpon Ehrenbergi*, *Sclerotinia sativa*, and *Fusarium avenaceum*. Similarly, the fungi that attack growing plants of sweet clover are *Phytophthora Cactorum*, *Fusarium culmorum*, *F. avenaceum*, and *S. sativa*. Alfalfa was markedly more resistant than sweet clover to all these fungi, except the low-temperature basidiomycete.

Of the alfalfa varieties tested for resistance to *C. Ehrenbergi* and *S. sativa*, *Medicago falcata* stood highest. Cossack and Viking ranked somewhat below *M. falcata*, but probably only slightly above the other varieties, which included Grimm.

The data from alfalfa tests in naturally infested soil suggest that *Medicago falcata*, Cossack and Viking may possess some resistance to the low-temperature basidiomycete. However, final conclusions must await further study. Present indications are that all varieties of sweet clover are highly susceptible to this pathogen.

Of the sweet clover varieties tested for resistance to *S. sativa*, those belonging to *M. alba* were more susceptible than those of *M. officinalis*. Redfield Yellow (*M. suaveolens*) was only slightly less susceptible than the varieties of *M. alba*. This variety and Arctic (*M. alba*) were the only ones that appeared to possess some resistance to *C. Ehrenbergi*. Inconclusive results were obtained with respect to *F. avenaceum*.

A strain of Alpha sweet clover (developed at Saskatoon) was quite resistant to *P. Cactorum*. The original Alpha variety was moderately susceptible. Arctic and all other varieties tested were highly susceptible.

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PRODUCTION AND VALUE OF POLYPLOID FIELD ROOTS[1]

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With the discovery of the value of colchicine for inducing chromosome doubling in plant species considerable success has been attained by many investigators in obtaining new tetraploid forms. The practical value of such plant forms can only be determined by trial since it cannot be predicted beforehand whether doubling the chromosome number of a particular economic crop plant will result in a favourable modification of such characters as yield, resistance to the environmental extremes of temperature and moisture, and chemical constitution.

This paper reports the work carried on at this Division during the past three years in which tetraploid and triploid forms of the three field root crops, sugar beets, mangels and swedes were obtained and their yields compared in field trials.

Tetraploid sugar beets have been successfully produced by Frandsen (3), Rasmusson and Levan (9), Schwanitz (11) and Peto and Boyes (7). By crossing the tetraploid with the diploid the latter investigators produced triploids and compared their yielding ability with that of diploids.

MATERIALS AND METHODS

Colchicine Treatment and Identification of C-Plants

The varieties used were Cesina sugar beets, Tip Top mangels and Laurentian swedes. Cesina, which was supplied by the Canada and Dominion Sugar Co., Ltd., is highly resistant to Cercospora. Tip Top mangel is of the yellow intermediate type and is relatively high in dry matter; this variety was developed by the Division of Forage Plants, Ottawa. Laurentian swede, produced by MacDonald College, Que., is highly regarded as a feed and table swede in areas where clubroot is not a serious limiting factor.

The seed treatment method was used with all three crops. It was found with swedes that a direct treatment of the seeds for 24 hours in a .2 to .4% aqueous solution of colchicine was effective in producing the characteristic symptoms of polyploidy. In sugar beet and mangels, however, owing to the seeds being embedded in a corky perianth it was necessary to presoak them in water for several days until the radicles and plumules had emerged. These germinated seeds were then treated with a .2 to .4% colchicine solution for 2 to 3 hours. The seeds were then washed and planted in flats in the greenhouse. After a month in flats the C-plants (those that showed colchicine effects) were pricked off into 3-inch pots. The characters that were used to identify the C-seedings at this

[1] Contribution from the Division of Forage Plants, C.E. Farm, Ottawa.
[2] Cytologist.

stage were degree of leaf thickening and crinkling and diameter of the taproot. In C-plants the leaves were thicker and more crinkled, and the taproot was thicker than in check plants. The colchicine treatment also had a retarding effect on growth in the early stages.

In early May, 1941, the seedlings were set out in a nursery adjacent to the greenhouse. This nursery material consisted of 120 C-plants and 10 checks of Cesina; 97 C-plants and 10 checks of Tip Top, and 137 C-plants and 10 checks of Laurentian.

During the summer the stomatal size on typical leaves of all the plants was measured. This was done by stripping a piece of epidermis from the lower surface of the leaf and mounting it in a staining medium of glycerine and 50% alcohol (1 : 1) to which a few drops of safranin were added. The stomatal size was found to be very similar in all three species. In the diploids the length ranged from .024 to .028 mm. while others which were considered to be tetraploids ranged from .036 to .048 mm. Values in between and admixtures of the two extremes were frequently found and were probably plants in which a transition from the tetraploid to the diploid condition was taking place. Plate I, 1 and 2, illustrates difference in stomatal size in swedes.

Hybridization of Diploids and Tetraploids

The roots were harvested in early October. Twelve roots of each variety were selected to be grown in the greenhouse for hybridization work. Ten out of each 12 were roots the stomatal measurements of which appeared to place them in the tetraploid class; the other 2 were checks. These roots were tagged and stored in the root cellar for a 2-months resting period. On December 15 they were planted in the greenhouse in 3-gallon jars.

The plants grew well and by the use of artificial light they were brought to flower by March. They flowered in the order, swedes, sugar beets, mangels. A few plants in each group flowered too late to be of any use in crossing work. Some abnormal plants also occurred which were not used (Plate II, 10). Other plants classed as tetraploids in the field reverted to the diploid condition in the greenhouse. Rasmusson and Levan (9) encountered the same difficulty of plants reverting to diploidy if partially changed into tetraploidy. Before using a plant in crossing work its status as diploid or tetraploid was established by pollen measurements. The difference in the size of the pollen was not as marked as in the case of stomata, but since the relative size is proportional to the cube of the radius, a small measured difference in the diameter indicates a much greater difference in the actual volume of the two types of pollen. Pollen from diploids ranged in size from .035 to .042 mm. for all three varieties of roots while that of tetraploids varied from .04 to .05 mm. (Plate I, 3 and 4). In the case of mangel and sugar beet, pollen from tetraploids appeared quite viable with only a small amount of shrunken, empty or defective grains. The pollen from the tetraploid swedes, however, had about 50% defective grains. This would indicate greater irregularity in the meiosis of the 4n-swede than in the 4n- Beta species.

The crossing technique used in the three crops differed somewhat on account of differences in flower structure. With the swedes it was relatively simple to emasculate and pollinate, using good control methods.

On the other hand, it was extremely difficult to emasculate mangels and beets without injury to the delicate stigma and style which resulted in little or no seed upon pollination. One method largely followed was to collect pollen from the male parent in a petri dish and dust it generously into the racemes of the female parent at daily intervals. It was assumed that the foreign pollen would be successful in competition with self pollen. Another method used was to place the two plants to be crossed close together and shake them once daily during the flowering period. In a test of foreign versus self pollen, a plant of the Tip Top variety was paired in this manner with one of the Prince variety. The seed set on the Tip Top plant was used in 1942 in the replicated plot test and the resulting plants all proved to be hybrids.

The results of the seed setting from the crossing and selfing of the swede plants are given in Table 1. The selfed and crossed diploid racemes gave seeds with about the same number of seeds in each—approximately 20 seeds per pod. The pods containing triploid seed gave the low number of 1.7 seeds per pod. If the cross was made with the diploid as the female, the pods were normal in appearance but contained mainly non-viable and shrunken seed. The tetraploid selfs and crosses produced very short pods with three to four seeds per pod. These seeds differed from the diploids in two respects. They were generally larger and about 20% of them were in an arrested stage of germination (Plate II, 2). These seeds proved to be quite capable of continued germination and normal development upon planting.

Table 1.—Seed setting in swedes

Cross or self	No. of pods	No. of seeds	Seeds per pod
Diploid self	25	555	22.2
Diploid cross	61	1170	19.2
Triploid	54	91	1.7
Tetraploid self	75	221	2.9
Tetraploid cross	121	427	3.5

RESULTS OF YIELD TRIALS

The object of the crossing was to secure enough seed of the different chromosome types to conduct replicated field trials with the following material of each variety: check (commercial seed), diploid self, diploid cross, triploid, tetraploid self and tetraploid cross. To conserve the seed as far as possible it was sown in trays, the resulting seedlings then pricked off in pots and later transplanted to the field.

The seeding was done May 15 and the transplanting on June 16. In the sugar beet and swede test each plot consisted of a row 34 feet long with the plants spaced 1 foot apart and the rows 3 feet apart. The outer rows in the test were filler or guard rows. In the sugar beets there was enough material for four replicates, except the diploid cross and tetraploid

cross where three replicates were used. In the swedes there were enough plants for five replicates, except the triploid (2 plots) and diploid cross (4 plots). For the mangel test there were insufficient plants for 34-foot rows so the plots were made 18 feet in length and five replicates used except the diploid cross (4 plots) and tetraploid cross (one plot).

The weather in late June was very favourable for the establishment of the young seedlings and the stands were quite good for the mangels and sugar beets but the tetraploid swedes suffered severely because of their low vigour. While prolonged periods of dry weather were unfavourable to growth, the stands were in general good at harvest time and the roots of fair size. The sugar beets were quite free from Cercospora. The swedes in September were badly infested with apids although this could not be considered a factor in reducing the yield of any particular type.

The roots were harvested and weighed individually the week of October 6 to 11. In sampling for dry matter 10 roots were taken from each plot. These were then divided into two lots of 5. Each root was quartered longitudinally and a quarter from each root sliced thinly and used to make up a weighed sample. The duplicate samples were then dried down in the dehydrator to absolute dry weight, weighed again, the percentage dry matter determined and the duplicates averaged and used as the percentage dry matter for the plot. This sampling technique gave quite uniform results as shown by Table 2, in which the dry matter percentages of the sugar beets are given.

Table 2.—Dry matter percentages in sugar beets

Material	Replicates								
	I		II		III		IV		
	D.M.	Ave.	D.M.	Ave.	D.M.	Ave.	D.M.	Ave.	Mean
Check	27.0 24.4	25.7	25.1 24.1	24.6	23.7 23.2	23.5	23.8 23.6	23.7	24.4
Diploid self 2 n s	21.3 22.2	21.7	22.3 21.3	21.8	23.5 23.7	23.6	22.8 23.8	23.3	22.6
Diploid cross 2 n c	21.8 21.5	21.6	22.1 22.2	22.15	23.2 23.3	23.3		22.0*	22.3
Triploid 3 n	20.2 21.9	21.05	23.3 22.6	22.9	24.3 21.9	23.1	23.3 22.7	23.0	22.5
Tetraploid self 4 n s	23.5 22.3	22.9	23.7 28.5	26.1	23.6 23.4	23.5	22.8 21.5	22.15	23.4
Tetraploid cross 4 n c	22.0 22.4	22.2	22.4 20.8	21.6	23.7 22.5	23.2		22.0*	22.3

* Filled by missing plot method.

Very seldom do the duplicates differ by more than 1%. The analysis of variance applied to the above data showed no significant difference in the dry matter percentage of each type. This point will be referred to later when the sugar content is considered.

End roots and roots on each side of a miss in a plot were discarded in calculating the yield per plot. The total weight of the remaining roots was used as a basis for determining the yield from a perfect stand. This is expressed in the tables in terms of tons, dry weight per acre for mangels and swedes, and tons per acre green weight for sugar beets.

In Table 3 the results from the sugar beet test are presented. The fourth replicates of the diploid and tetraploid crosses were filled by the missing plot method, devised by Allen and Wishart (1). This requires the deduction of two degrees of freedom in the analysis of variance. This analysis showed that the variance due to varieties was significant with a necessary difference between the means of the varieties of 1.35 tons per acre. The chief contribution to this significance appears to be the superior yield of the two diploids (cross and self) over the check and tetraploid. The triploid occupies an intermediate position in yield between diploid and tetraploid although it is not significantly different from either.

TABLE 3.—GREEN WEIGHT OF SUGAR BEETS IN TONS PER ACRE

Material	Replicates					
	I	II	III	IV	Total	Mean
Check	13.88	12.41	13.99	14.64	54.92	13.76
Diploid self	16.07	16.35	17.42	15.62	65.46	16.37
Diploid cross	14.65	13.71	17.02	14.72*	60.10	15.03
Triploid	14.15	14.56	14.99	14.52	58.22	14.56
Tetraploid self	15.00	11.96	15.23	12.31	54.50	13.63
Tetraploid cross	14.11	13.45	13.32	13.21*	54.09	13.52
Total	87.86	82.44	91.97	85.02		14.47
Necessary difference for significance	2.71	2.71	2.71	2.71		1.35

* Filled by missing plot method.

TABLE 4.—DRY WEIGHT OF MANGELS IN TONS PER ACRE

Material	Replicates						
	I	II	III	IV	V	Total	Mean
Check (Tip Top)	3.28	3.84	3.46	2.82	3.32	16.72	3.34
Diploid self	3.45	3.44	3.29	2.41	2.84	15.43	3.09
Diploid cross	3.92	4.55	4.72	3.73	4.41*	21.33	4.27
Triploid	3.57	3.16	3.79	3.04	3.04	16.60	3.32
Tetraploid self	2.84	2.54	2.91	2.68	2.89	13.86	2.77
Tetraploid cross	2.13						
Total	17.06	17.53	18.17	14.68	16.50	83.94	2.36
Necessary difference for significance	0.83	0.83	0.83	0.83	0.83		0.37

* Filled by missing plot method.

Table 4 gives the results of the mangel test. The fifth replicate of the diploid cross was filled by the missing plot method. Very little seed was secured from the tetraploid cross and the yield of the single plot grown is given in the table although it was not included in the analysis of variance. The analysis showed that variance due to varieties was significant with a necessary difference of .37 tons per acre. Plants from the cross Tip Top × Prince yielded significantly better than the Tip Top check or its polyploid derivatives. While this material is not strictly comparable to the polyploid series it does show that the utilization of varietal crosses with the resulting hybrid vigour and uniformity is a more effective means of increasing yield in mangels than by increasing the chromosome complement.

TABLE 5.—DRY WEIGHT OF SWEDES IN TONS PER ACRE

Material	Replicates						
	I	II	III	IV	V	Total	Mean
Check (Laurentian)	1.38	1.38	0.82	0.92	1.06	5.56	1.11
Diploid self	1.44	1.48	0.93	0.82	1.36	6.03	1.21
Diploid cross	1.68	1.08	0.90	0.77	1.58	6.01	1.20
Triploid	1.19	0.46					
Tetraploid self	1.23	0.46	0.40	0.31	0.43*	2.83	0.57
Tetraploid cross	0.39	0.29	0.19	0.23	0.31	1.41	0.28
Total	6.12	4.69	3.24	3.05	4.74	21.84	0.87
Necessary difference for significance	0.59	0.59	0.59	0.59	0.59		0.27

* Filled by missing plot method.

Table 5 gives the yield results of the swedes. The small amount of triploid seed secured made a statistical treatment of its yield impossible although the yield from the two plots grown are given in the table. An analysis of variance showed the difference due to varieties was highly significant. The check and diploids were markedly superior to the tetraploids in yield. Typical roots are illustrated in Plate II, 2. Swedes with a somatic chromosome number of 36 are apparently more adversely affected by doubling the chromosome number than mangels and sugar beets with a chromosome number of 18.

THE SUGAR CONTENT OF SUGAR BEETS

Table 6 gives the data supplied by the Division of Chemistry on the roots which were submitted for sugar analysis. The weight in grams of the trimmed and untrimmed roots and the dry matter percentage is also given in the table. In selecting the roots for analysis two were taken from each plot differing more or less in size. The mean root size within each group was fairly similar being 1100 to 1200 grams. The trimmed roots weighed approximately 60% as much as the untrimmed roots. This loss of 40% is rather high but two factors contributed to it. The root prongs were somewhat twisted and interlaced on account of transplanting and they required drastic pruning to ensure that they were free from earth before sampling for sugar. Secondly the plants were topped leaving an

inch of leaf petiole since the balance of the polyploids not used in sampling was pitted in case their use for stecklings in 1942 was advisable. Before sampling the root was trimmed to the lowest leaf scar.

TABLE 6.—WEIGHT, DRY MATTER AND SUGAR CONTENT OF SUGAR BEETS

Material	No. of roots	Weight of roots		Percentage original basis	
		Untrimmed gm.	Trimmed gm.	Dry matter	Total sugar
Check	14	1104	657	26.5	18.0
Diploid cross	6	1158	679	26.5	18.78
Diploid self	10	1150	762	24.6	17.53
Triploid	10	1134	647	26.1	18.03
Tetraploid cross	6	1162	676	24.8	18.31
Tetraploid self	10	1329	835	24.8	17.66

The mean sugar content varied but slightly from group to group ranging from 17.53 to 18.78%. It may be noted that both crossed diploids and tetraploids had a higher sugar content than their respective selfs. The mean root size of the sampled roots was approximately the same in the diploid crosses and selfs, although the tetraploid selfs were somewhat larger than the tetraploid crosses. This approximate similarity of sugar content is in agreement with the dry matter percentages given in Table 2. This is to be expected since in Table 8 it is shown that there is a positive correlation between percentage dry matter and percentage sugar.

TABLE 7.—SUGAR YIELD OF POLYPLOID SUGAR BEETS IN TONS PER ACRE—1941

Strain	Replicates					
	I	II	III	IV	Total	Mean
Check	1.51	1.35	1.52	1.59	5.97	1.49
Diploid self	1.69	1.72	1.83	1.64	6.88	1.72
Diploid cross	1.65	1.54	1.92	1.66	6.77	1.69
Triploid	1.53	1.58	1.62	1.57	6.30	1.58
Tetraploid self	1.59	1.27	1.61	1.30	5.77	1.44
Tetraploid cross	1.58	1.48	1.46	1.45	5.94	1.48
Total	9.52	8.94	9.96	9.21	37.63	1.57
Necessary difference for significance	0.29	0.29	0.29	0.29		0.15

The yield of sugar per acre given in Table 7 is based on the green yield and the mean sugar content for each polyploid group. The same differences are apparent as in the green yield. The diploid cross and self were significantly better than the check and tetraploid with the triploid being intermediate in yield but not significantly different from the two extremes.

The degree of correlation existing between the percentage sugar and green weight and percentage dry matter is given in Table 8. These calculations are based on the data of Table 6 with the green weight of the trimmed roots being used.

TABLE 8.—CORRELATION OF SUGAR CONTENT WITH DRY MATTER PERCENTAGE AND GREEN WEIGHTS IN SUGAR BEETS

Characters correlated	No. of plants	r
Green weight and percentage sugar of all roots	56	− 0.318
Green weight and percentage sugar of diploids and check	30	− 0.199
Green weight and percentage sugar of triploids and tetraploids	26	− 0.304
Dry matter and percentage sugar of all roots	56	0.493
Dry matter and percentage sugar of diploids and checks	30	0.339
Dry matter and percentage sugar of triploids and tetraploids	26	0.801

All values of r are significant as measured by Fisher's (2) V.A. table. Green weight and sugar content are seen to be negatively correlated. The correlation in the case of the triploids and tetraploids is somewhat higher than for the diploids. Various workers have found sugar content and green weight to be correlated. Pack (5) found this correlation to vary in different lines while Garner and Saunders (4) found it to be influenced by environmental conditions such as spacing and amount of rainfall. Peto and Boyes (7) in their material found the r value for diploids to be -0.74 and for triploids -0.54 which they interpreted to mean that percentage of sugar does not decrease with increased weight of root as rapidly in triploids as in diploid beets. With our material the difference in the respective values cannot be considered as significant.

VARIABILITY IN ROOT WEIGHT

Since all roots were weighed individually it was possible to determine the uniformity of the various lots. Roots at the ends of the rows and at either side of a blank were not included in deriving the following data on uniformity.

TABLE 9.—MEAN GREEN WEIGHT (OZ.) AND COEFFICIENT OF VARIABILITY FOR THE VARIOUS POLYPLOID ROOTS

Material	Sugar beets		Mangels		Swedes	
	Mean wt.	C.V.	Mean wt.	C.V.	Mean wt.	C.V.
Checks	30.3 ± 1.03	34.0	45.4 ± 2.27	39.9	30.0 ± 1.87	49.0
Diploid self	36.9 ± 0.94	25.3	42.6 ± 1.96	36.8	30.5 ± 1.41	47.0
Diploid cross	33.0 ± 1.43	40.0	72.1 ± 2.23	24.6	29.5 ± 1.57	53.2
Triploid cross	33.3 ± 1.51	45.2	43.0 ± 2.19	40.9		
Tetraploid self	26.3 + 1.62	61.8	43.8 + 2.46	45.0	17.0 + 1.73	86.0
Tetraploid cross	30.1 ± 1.34	43.1			7.0 ± 0.65	80.0

The mean yields very closely reflect the yield results given in Tables 3 to 5. It may be seen that the triploids and tetraploids were more variable than the checks and diploids. The diploid cross in mangels gave a very uniform lot of roots. The roots were high in green weight although the absolute dry weight is relatively reduced since the percentage dry matter is 12.9 as compared to 15.9 for the Tip Top check.

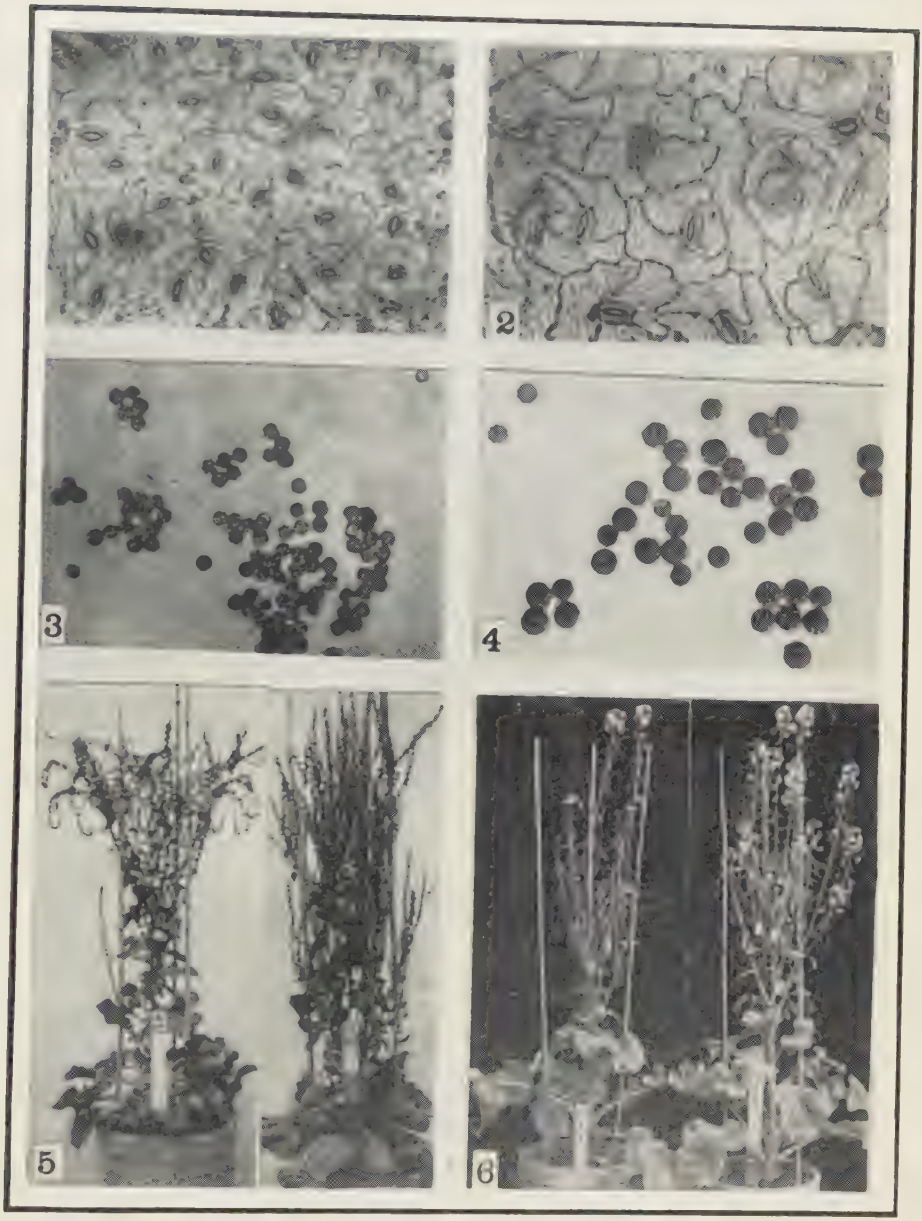

PLATE I. 1. Stomata of diploid swede. 2. Stomata of tetraploid swede. 3. Pollen of diploid sugar beet. 4. Pollen of tetraploid sugar beet. 5. Diploid sugar beet left and tetraploid right; 6. Diploid swede left and tetraploid right.

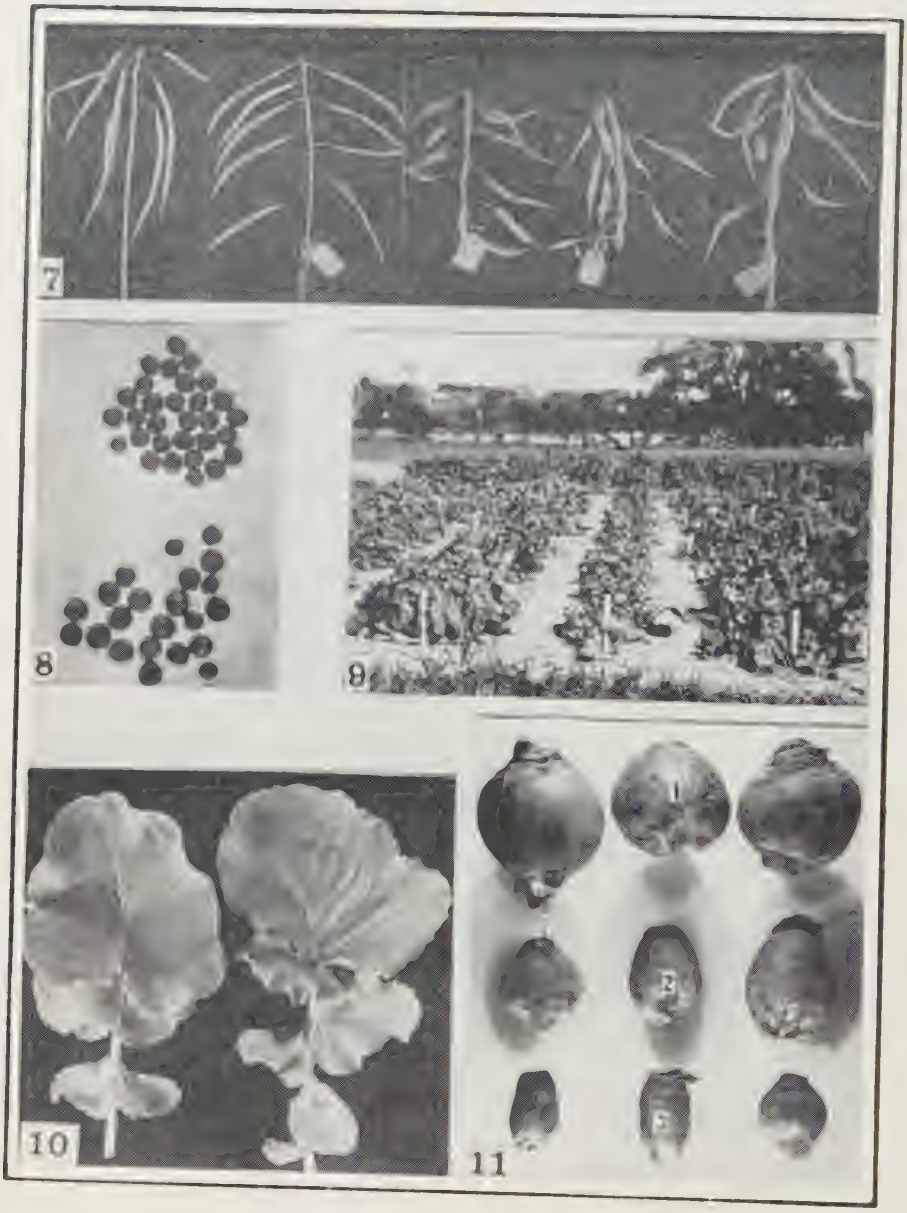

PLATE II. 7. Pods on swede crosses. Left to right diploid cross, diploid self, tetraploid cross, tetraploid self and triploid. 8. Swede seeds: diploid above, tetraploid below. 9. Swede yield test: diploid cross left; tetraploid cross centre; check right. 10. Normal leaf of swede left; chimaera right. 11. Mature roots of swede; checks above; triploid centre; tetraploid below.

DISCUSSION

The yield trials reported here tend to show that no results of practical value were gained by doubling the chromosome complement in the varieties of field roots tested. In the swedes the tetraploids were distinctly inferior to the diploids, the yield being less than one-half. Triploid swedes were very hard to obtain, the cross diploid × tetraploid being highly sterile. In the mangels and sugar beets the effect was not so marked, the triploids being equal to the checks in yield although lower than selected diploids; the tetraploids were significantly lower in yield than the diploids.

Several workers have reported advantages accruing from tetraploidy other than yield. Randolph and Hand (8) found that tetraploids strains of yellow corn were higher in carotinoids than the diploids. Tetraploid *Lolium perenne* was found by Sullivan and Myers (12) to contain more reducing sugar and sucrose then the diploid material. According to Sansome and Zilva (10) tetraploid strains of tomato were higher than diploids in vitamin C. In the case of the sugar beet variety investigated here there was no increase in the percentage sugar in triploids and tetraploids over diploids. The degree of correlation between green weight and sugar content indicated that sugar content decreased at about the same rate with increase of root size in all types of polyploids. Working with the variety Great Western, Peto and Boyes (7) obtained not only an increase in green yield of triploid over diploid, but also a higher sugar content in the triploid. The difference in the r values of green weight and percentage sugar for diploids and triploids indicated that the selection of larger roots in the tetraploid might lead to less reduction in sugar content than similar selection in diploids.

It is evident, however, that considering the work entailed in producing tetraploid strains, maintaining stocks, and producing seed in commercial quantities, triploids and tetraploids should show a substantial increase in yield over diploids to warrant this extra work.

The creation of autotetraploids usually leads to characteristic meiotic disturbances. Sugar beet tetraploids examined by Rasmusson and Levan (9) showed 0–4 quadrivalents in the first meiotic division. While this irregularity was not severe enough to prevent the formation of a high percentage of good pollen yet it would lead to irregular chromosome numbers for several generations with a tendency to revert to the diploid condition.

While it is apparent that all attempts to create new and improved autotetraploid strains may not meet with success especially in those species which already are polyploid in type, the attempt is worth while with any important economic crop. There is also a promising field of work in the production of allopolyploids. There is a considerable mass of evidence to show that many of the valuable plant species are allopolyploids originating from interspecific or intergeneric crosses resulting in the summation of two distinct chromosome sets frequently followed by chromosome doubling. The value of colchicine in bringing about this last step is illustrated by Peto and Boyes (6) who converted the sterile hybrid, *Triticum dicoccum* × *Agropyron glaucum* into a fertile amphidiploid.

SUMMARY

1. Tetraploid plants of the three field root crops, sugar beets, mangels and swedes were obtained by means of colchicine treatments of germinating seed.

2. In the winter of 1940—41 stecklings of these tetraploids were grown in the greenhouse and crossed *inter se* and with diploids. Enough seed of the following chromosome types in each variety was available for yield trials in 1942: check, diploid cross, diploid self, triploid, tetraploid cross and tetraploid self.

3. The seedlings were started in pots May 15 and transplanted to the field on June 18. The plots were replicated four or five times in each test.

4. Harvesting was completed in early October. The roots were weighed individually, sampled for dry matter and the sugar beets were analyzed for sugar content.

5. The analyses of variance showed significant differences in yield in all three trials. Diploids yielded significantly better than tetraploids and triploids were intermediate in yield.

6. The various sugar beet chromosome types showed no significant differences in percentages dry matter or sugar content.

7. Green weight and percentage sugar in sugar beets were found to be negatively correlated, while dry matter and sugar content were positively correlated.

8. It was concluded that in the case of the varieties of field roots investigated the development of tetraploid strains would serve no practical purpose.

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SUBJECT INDEX TO VOLUME XXII

Page

Advantages of a soil paste for routine pH determination, The 135
Agropyron cristatum L. in controlling perennial weeds, The place of crested wheat grass 459
Alfalfa and sweet clover to root- and crown-rotting fungi in Alberta, Varietal resistance of 775
Alfalfa Seed setting in. II 129
Alternaria blotch of wheat. Brown necrosis and 746
Analysis of covariance by the method of individual comparisons, The 721
Apical leaf speck of potatoes 772
Apple mealybug *Phenacoccus aceris* Sig. and its control by dormant sprays, The ... 727
Apricots. Boron deficiency in 271

Bacon hogs, The feeding value of Canadian Western grains for, I. and II 326
Bacteriophage of rhizobia, Studies with the 209
Barley. Cultural studies with
I. Differential responses of varieties to date of seeding with respect to yield 225
II. Differential responses to fertilizer treatment and rate of seeding with respect to yield 659
Barleys, Malting quality of Canadian. III. Twenty-eight varieties, 1938, 1939, and 1940 trials 584
Barleys, Prediction of malt diastatic power of hybrid 761
Book reviews 68, 125, 208, 274, 519, 645, 725
Boron deficiency in apricots 271
Brown necrosis and alternaria blotch of wheat 746
Butter, Studies on surface taint.
I. Odour production by *Pseudomonas putrefaciens* 277
II. An odorous compound in skim milk culture of *Pseudomonas putrefaciens* 347
III. Some further characteristics of *Pseudomonas putrefaciens* 438
IV. Distribution and taxonomy of *Pseudomonas putrefaciens* 461
V. The growth of *Pseudomonas putrefaciens* in butter 552
VI. Other bacterial species as causal agents. *Flavobacterium maloloris* (n. sp.) .. 637

Calyx spray in codling moth control, The 413
Carnations, Sleepiness in 509
Cereal crops in Saskatchewan, Some observations on the tolerance of salinity by ... 492
Chemical weed control in lawns 356
Cobalt deficiency symptoms in sheep, The efficacy of cobaltized salt for the prevention of 479
Codling moth control, The calyx spray in 413
Codling moth control in British Columbia, Recent progress in
I. Killing the mature larva 571
II. Killing the adult 577
Comparative study of the influence of temperature on the development of certain sawflies after hibernation in the cocoon, A 298
Corn studies. Hybrid. I 307
Covariance by the method of individual comparisons, The analysis of 721
Cultural studies with barley.
I. Differential responses of varieties to date of seeding with respect to yield 225
II. Differential responses to fertilizer treatment and rate of seeding with respect to yield 659

60368

Page

Dairy products in the City of Guelph, Ontario, A note on the consumption of..... 410
Development of cobalt deficiency in sheep, The..... 314
Development of the lily..... 104
Digestibility studies with foxes.
I. Effect of the plane of nutrition upon the digestibility of meats..... 18
II. Digestibility of frozen beef tripe, frozen lip meat, frozen beef hearts and frozen cow udders..... 33
Digestibility studies with ruminants.
VIII. Associative digestibility of hay and grains..... 250
IX. Associative digestibility of grains: wheat bran, gluten feed and soybean oil meal..... 561

Economic aspects of poultry keeping in Canada. II. Relationships between prices of feed and eggs and their effect on the return to the poultryman for labour and management..... 448
Effect of ammonium phosphate on the yield and quality of linseed flax in northern Saskatchewan and northeastern Alberta, The..... 195
Effect of fertilizer treatments on the yield and chemical composition of pasture species, The..... 733
Effect of stem rust on the yield, quality, chemical composition, and milling and baking properties of Marquis wheat, The..... 40
Effects of wind erosion on the composition and fertility of some Alberta soils..... 533
Efficacy of cobaltized salt for the prevention of cobalt deficiency symptoms in sheep, The..... 479

Feeding value of Canadian western grains for bacon hogs, The I. and II..... 326
Feed requirements of market hogs, The..... 390
Fertilizer treatments on the yield and chemical composition of pasture species, The effect of..... 733
Field roots, production and value of polyploid..... 787
Flavobacterium maloloris (n. sp.). Studies on surface taint butter. VI. Other bacterial species as causal agents..... 637
Flax in northern Saskatchewan and northeastern Alberta, The effect of ammonium phosphate on the yield and quality of linseed..... 195

Millet, The inheritance of plant colour and the extent of natural crossing in foxtail 80

Necrotic "fern-leaf" mosaic of raspberry, A..... 119
Note on the consumption of dairy products in the City of Glelph, Ontario, A..... 410

Oat varieties, Root development in..... 503
Observations on antagonism in inoculation tests of wheat with *Helminthosporium sativum* P.K. & B., and *Fusarium culmorum* (W.G. Sm.) Sacc..... 688

Pasture species, The effect of fertilizer treatments on the yield and chemical composition of..... 733
Pasture studies.
XX. An ecological and crop survey of Stanstead county..... 178
XXII. Dry matter defecation as an index of forage intake by grazing steers..... 242
pH determination, The advantages of a soil paste for routine..... 135
Phenacoccus aceris Sig. and its control by dormant sprays, The apple mealybug..... 727
Place of crested wheat grass, *Agropyron cristatum* L. in controlling perennial weeds, The..... 459
Polypoloid field roots, production and value of..... 787
Potatoes, Apical leaf speck of..... 772
Poultry; Further data on the relation between shell strength, potential hatchability and chick vitality in the fowl..... 698

Page

Poultry keeping in Canada, Economic aspects of. II. Relationship between prices of feed and eggs and their effect on the return to the poultryman for labour and management ... 448
Poultry, The relative effect of environment and heredity upon body measurements and production characteristics in
I. Period of growth ... 378
II. Period of egg production ... 482
Prediction of malt diastatic power of hybrid barleys ... 761
Predominance of race 56 in relation to the stem-rust resistance of Ceres wheat, The ... 152
Prevalence of seed-borne fungi on cereals in certain seed inspection districts of Canada ... 419
Production and value of polyploid field roots ... 787
Pseudomonas, Requisites for the recognition of the blue-green ... 401
Pseudomonas putrefaciens, An odorous compound in skim milk cultures of ... 347
Pseudomonas putrefaciens, Distribution and taxonomy of ... 461
Pseudomonas putrefaciens in butter, The growth of ... 552
Pseudomonas putrefaciens, Odour production by ... 277
Pseudomonas putrefaciens, Some further characteristics of ... 438
Pythium root rot of grasses ... 674

Raspberry, A necrotic "fern leaf" mosaic of ... 119
Raspberry soils of British Columbia, Studies on some ... 287
Recent progress in codling moth control in British Columbia
I. Killing the mature larva ... 571
II. Killing the adult ... 577
Relation of soil organic matter to the production of flue cured tobacco, The ... 546
Relative effect of environment and heredity upon body measurements and production characteristics in poultry, The
I. Period of growth ... 378
II. Period of egg production ... 482
Removal of moisture from honey ... 157
Reproductive capacity of breeds of range sheep, The ... 11
Requisites for the recognition of the blue-green *Pseudomonas* ... 401
Responses of tobacco seedlings to chemical growth substances ... 528
Rhizobia, Studies with the bacteriophage of ... 209
Root development in oat varieties ... 503
Root fibre production of some perennial grasses ... 108

Sawflies after hibernation in the cocoon, A comparative study of the influence of temperature on the development of certain ... 298
Seed-borne fungi on cereals in certain seed inspection districts of Canada, Prevalence of ... 419
Seedling resistance of wheat varieties to artificial drought in relation to grain yield, The ... 521
Seed setting in alfalfa. II ... 129
Sheep; Cobalt deficiency symptoms in, The efficacy of cobaltized salt for the prevention of ... 479
Sheep, The development of cobalt deficiency in ... 314
Sheep, The reproductive capacity of breeds of range ... 11
Sleepiness in carnations ... 509
Soil profiles from southern Alberta, Mechanical composition of some characteristic ... 341
Soils, Effects of wind erosion on the composition and fertility of some Alberta ... 533
Soils of Eastern Ontario, The ... 608
Soil, Some factors affecting the nitrate content of an orchard ... 61
Some factors affecting the nitrate content of an orchard soil ... 61
Some observations on the tolerance of salinity by cereal crops in Saskatchewan ... 492

Page

Soxhlet type apparatus for the simultaneous extraction of a large number of samples, A 337
Stanstead County, An ecological and crop survey of, Pasture studies XX 178
Studies on some raspberry soils of British Columbia 287
Studies on surface taint butter
I. Odour production by *Pseudomonas putrefaciens* 277
II. An odorous compound in skim milk cultures of *Pseudomonas putrefaciens* 347
III. Some further characteristics of *Pseudomonas putrefaciens* 438
IV. Distribution and taxonomy of *Pseudomonas putrefaciens* 461
V. The growth of *Pseudomonas putrefaciens* in butter 562
VI. Other bacterial species as causal agents. *Flavobacterium maloloris* (n. sp.) .. 637
Studies on the thermal resistance of honey yeasts 705
Studies with the bacteriophage of rhizobia 209
Study of the chlorphyll, xanthophyll and carotene contents of the wheat kernel harvested at successive stages of development, A 93
Study of the inheritance of solid stems in some tetraploid wheats, A 594
Sweet clover, Investigations concerning the coumarin content of. III. The inheritance of low coumarin character 85
Sweet clover to root- and crown-rotting fungi in Alberta, Varietal resistance of alfalfa and 775

Thiamin content of Canadian wheat and flour, Investigations on the 366
Tobacco mosaic in Ontario and Quebec 465
Tobacco seedlings to chemical growth substances, Responses of 528
Tobacco, The relation of soil organic matter to the production of flue cured 546
Triticum vulgare, The inheritance of solid stem and certain other characters in crosses between varieties of 216

Vacuum fumigation for insect control 170
Varietal resistance of alfalfa and sweet clover to root- and crown-rotting fungi in Alberta 775

Weed control, Hydrogen-ion concentration and 647
Weed control in lawns, Chemical 356
Wheat and flour, Investigations on the thiamin content of Canadian 366
Wheat, Brown necrosis and alternaria blotch of 746
Wheat kernel at successive stages of development, A study of the chlorophyll, xanthophyll and carotene contents of the 93
Wheats, A study of the inheritance of solid stems in some tetraploid 594
Wheat, The effect of stem rust on the yield, quality, chemical composition, and milling and baking properties of Marquis 40
Wheat; The inheritance of solid stem and certain other characters in crosses between varieties of *Triticum vulgare* 216
Wheat, The predominance of race 56 in relation to the stem-rust resistance of Ceres. 152
Wheat varieties, The influence of some environmental factors in the expression of the solid stem character in certain 139
Wheat varieties to artificial drought in relation to grain yield, The seedling resistance of 521
Wheat with *Helminthosporium sativum* P.K. & B. and *Fusarium culmorum* (W. G. Sm.) Sacc. Observations on antagonism in inoculation tests of 688

AUTHOR INDEX

	Page
Armstrong, J. M.	1, 787
Ashton, G. C.	390
Atkinson, H. J.	287
Berkeley, G. H.	465
Bird, S.	448
Bowser, W. Earl	341
Bowstead, J. E.	314, 479
Bradt, O. A.	61
Brown, A. W. A.	298
Campbell, J. A.	250, 561
Chamberlain, G. C.	119
Chapman, L. J.	608
Cormack, M. W.	775
Crampton, E. W.	242, 326, 337, 390
Darroch, J. G.	216, 521
Daviault, L.	298
Davidson, W. M.	250, 561
DeLong, W. A.	69
Dennys, A. A.	577
Derick, R. A.	503
Dickson, G. H.	61
Doughty, J. L.	135
Dunkley, W. L.	347
Erdman, R. L.	533
Ferguson, W.	509
Fitzpatrick, R. E.	271
Frankton, C.	178
Gibson, D. R.	1
Greaney, F. J.	40, 419
Gutteridge, H. S.	378, 482
Hagborg, W. A. F.	746
Hamilton, D. G.	503
Heriot, A. D.	571
Hood, E. G.	277, 347, 438, 461, 552, 637
Horner, W. H.	85
Horton, H. A.	546
Hunter, G.	347
Inman, W. R.	18, 33
Jackson, S. H.	366
Jamieson, M. C.	401
Johnson, T.	152, 746
Katznelson, H.	209
Kemp, H. J.	216
Laidlaw, H. C.	225, 659
Ledingham, R. J.	688
Lejeune, A. J.	225, 659
MacArthur, Mary	104
MacGregor, W. C.	195
MacIsaac, F. M.	195
MacLachlan, J. D.	201, 307
MacVicar, R. M.	80
McEvoy, E. T.	528
McRostie, G. P.	307
Machacek, J. E.	419
Manning, K. R.	69
Marshall, J.	413, 492, 727
Martin, D. T.	410
Meredith, W. O. S.	225, 584, 659, 761
Minshell, W. H.	647
Monro, H. A. U.	170
Muir, G. W.	250, 561
Munro, S. S.	698
Newton, Margaret	152
Nowosad, F. S.	733
Olson, P. J.	129, 225, 659
O'Neil, J. B.	378, 482, 721
Parnell, H. R.	80
Pavlychenko, T. K.	459
Platt, A. W.	139, 216, 521
Purdy, T. L.	242
Putnam, D. F.	608
Putnam, L. G.	594
Rasmussen, K.	11
Raymond, L. C.	178
Robinson, C. H.	250, 561
Rowland, H.	584, 761
Sackville, J. P.	314, 479
Sallans, H. R.	584, 761
Sanford, G. B.	772
Silversides, W. H.	129
Sinclair, R. D.	314, 479
Smith, G. E.	18
Stephen, W. A.	157, 705
Stevenson, T. M.	108
Thornton, H. R.	277, 347, 438, 461, 552, 637
Vanterpool, T. C.	674
Waddell, D. B.	413
Watson, C. J.	250, 561
White, W. J.	85, 108
Whiteside, A. G. O.	40, 366
Wolochow, H.	277, 438, 461, 552, 637
Wood, C. R.	93
Woodbridge, C. G.	271
Woods, J. J.	356
Woodward, J. C.	40, 733
Wright, L. E.	287, 733